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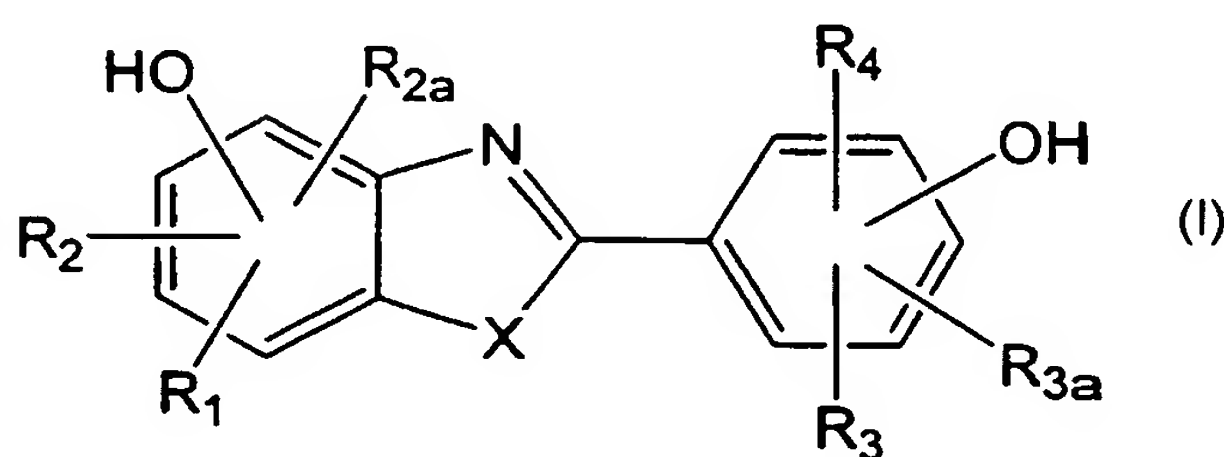
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(54) Title: SUBSTITUTED BENZOXAZOLES AND ANALOGUES AS ESTROGENIC AGENTS



(57) Abstract: This invention provides estrogen receptor modulators of formula (I), having the structure of formula (I) wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>2a</sub>, R<sub>3</sub>, R<sub>3a</sub>, and R<sub>4</sub>, and X as defined in the specification, or a pharmaceutically acceptable salt thereof.

## SUBSTITUTED BENZOXAZOLES AND ANALOGUES AS ESTROGENIC AGENTS

### BACKGROUND OF THE INVENTION

5 This invention relates to substituted benzoxazoles which are useful as estrogenic agents.

The pleiotropic effects of estrogens in mammalian tissues have been well documented, and it is now appreciated that estrogens affect many organ systems [Mendelsohn and Karas, New England Journal of Medicine 340: 1801-1811 (1999), Epperson, et al., Psychosomatic Medicine 61: 676-697 (1999), Crandall, Journal of  
10 Womens Health & Gender Based Medicine 8: 1155-1166 (1999), Monk and Brodaty, Dementia & Geriatric Cognitive Disorders 11: 1-10 (2000), Hurn and Macrae, Journal of Cerebral Blood Flow & Metabolism 20: 631-652 (2000), Calvin, Maturitas 34: 195-210 (2000), Finking, et al., Zeitschrift fur Kardiologie 89: 442-453 (2000), Brincat, Maturitas 35: 107-117 (2000), Al-Azzawi, Postgraduate Medical Journal 77: 292-304  
15 (2001)]. Estrogens can exert effects on tissues in several ways, and the most well characterized mechanism of action is their interaction with estrogen receptors leading to alterations in gene transcription. Estrogen receptors are ligand-activated transcription factors and belong to the nuclear hormone receptor superfamily. Other members of this family include the progesterone, androgen, glucocorticoid and  
20 mineralocorticoid receptors. Upon binding ligand, these receptors dimerize and can activate gene transcription either by directly binding to specific sequences on DNA (known as response elements) or by interacting with other transcription factors (such as AP1), which in turn bind directly to specific DNA sequences [Moggs and Orphanides, EMBO Reports 2: 775-781 (2001), Hall, et al., Journal of Biological  
25 Chemistry 276: 36869-36872 (2001), McDonnell, Principles Of Molecular Regulation. p351-361(2000)]. A class of "coregulatory" proteins can also interact with the ligand-bound receptor and further modulate its transcriptional activity [McKenna, et al., Endocrine Reviews 20: 321-344 (1999)]. It has also been shown that estrogen receptors can suppress NF $\kappa$ B-mediated transcription in both a ligand-dependent and  
30 independent manner [Quaedackers, et al., Endocrinology 142: 1156-1166 (2001), Bhat, et al., Journal of Steroid Biochemistry & Molecular Biology 67: 233-240 (1998), Pelzer, et al., Biochemical & Biophysical Research Communications 286: 1153-7 (2001)].

Estrogen receptors can also be activated by phosphorylation. This phosphorylation is mediated by growth factors such as EGF and causes changes in gene transcription in the absence of ligand [Moggs and Orphanides, EMBO Reports 2: 775-781 (2001), Hall, et al., Journal of Biological Chemistry 276: 36869-36872 (2001)].

A less well-characterized means by which estrogens can affect cells is through a so-called membrane receptor. The existence of such a receptor is controversial, but it has been well documented that estrogens can elicit very rapid non-genomic responses from cells. The molecular entity responsible for transducing these effects has not been definitively isolated, but there is evidence to suggest it is at least related to the nuclear forms of the estrogen receptors [Levin, Journal of Applied Physiology 91: 1860-1867 (2001), Levin, Trends in Endocrinology & Metabolism 10: 374-377 (1999)].

Two estrogen receptors have been discovered to date. The first estrogen receptor was cloned about 15 years ago and is now referred to as ER $\alpha$  [Green, et al., Nature 320: 134-9 (1986)]. The second form of the estrogen receptor was found comparatively recently and is called ER $\beta$  [Kuiper, et al., Proceedings of the National Academy of Sciences of the United States of America 93: 5925-5930 (1996)]. Early work on ER $\beta$  focused on defining its affinity for a variety of ligands and indeed, some differences with ER $\alpha$  were seen. The tissue distribution of ER $\beta$  has been well mapped in the rodent and it is not coincident with ER $\alpha$ . Tissues such as the mouse and rat uterus express predominantly ER $\alpha$ , whereas the mouse and rat lung express predominantly ER $\beta$  [Couse, et al., Endocrinology 138: 4613-4621 (1997), Kuiper, et al., Endocrinology 138: 863-870 (1997)]. Even within the same organ, the distribution of ER $\alpha$  and ER $\beta$  can be compartmentalized. For example, in the mouse ovary, ER $\beta$  is highly expressed in the granulosa cells and ER $\alpha$  is restricted to the thecal and stromal cells [Sar and Welsch, Endocrinology 140: 963-971 (1999), Fitzpatrick, et al., Endocrinology 140: 2581-2591 (1999)]. However, there are examples where the receptors are coexpressed and there is evidence from in vitro studies that ER $\alpha$  and ER $\beta$  can form heterodimers [Cowley, et al., Journal of Biological Chemistry 272: 19858-19862 (1997)].

A large number of compounds have been described that either mimic or block the activity of 17 $\beta$ -estradiol. Compounds having roughly the same biological effects

as 17 $\beta$ -estradiol, the most potent endogenous estrogen, are referred to as "estrogen receptor agonists". Those which, when given in combination with 17 $\beta$ -estradiol, block its effects are called "estrogen receptor antagonists". In reality there is a continuum between estrogen receptor agonist and estrogen receptor antagonist activity and indeed some compounds behave as estrogen receptor agonists in some tissues and estrogen receptor antagonists in others. These compounds with mixed activity are called selective estrogen receptor modulators (SERMS) and are therapeutically useful agents (e.g. EVISTA) [McDonnell, Journal of the Society for Gynecologic Investigation 7: S10-S15 (2000), Goldstein, et al., Human Reproduction Update 6: 212-224 (2000)]. The precise reason why the same compound can have cell-specific effects has not been elucidated, but the differences in receptor conformation and/or in the milieu of coregulatory proteins have been suggested.

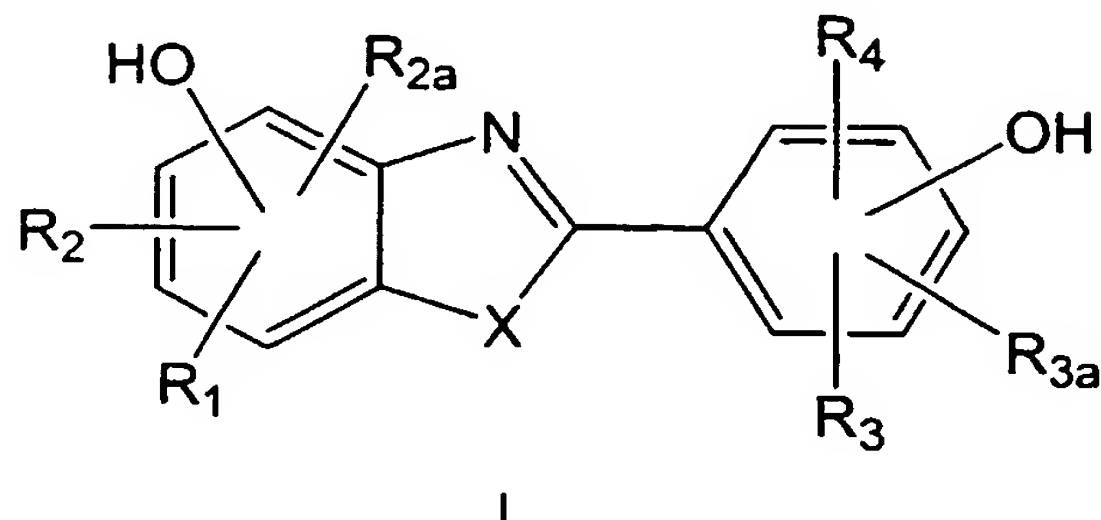
It has been known for some time that estrogen receptors adopt different conformations when binding ligands. However, the consequence and subtlety of these changes has been only recently revealed. The three dimensional structures of ER $\alpha$  and ER $\beta$  have been solved by co-crystallization with various ligands and clearly show the repositioning of helix 12 in the presence of an estrogen receptor antagonist which sterically hinders the protein sequences required for receptor-coregulatory protein interaction [Pike, et al., Embo 18: 4608-4618 (1999), Shiau, et al., Cell 95: 927-937 (1998)]. In addition, the technique of phage display has been used to identify peptides that interact with estrogen receptors in the presence of different ligands [Paige, et al., Proceedings of the National Academy of Sciences of the United States of America 96: 3999-4004 (1999)]. For example, a peptide was identified that distinguished between ER $\alpha$  bound to the full estrogen receptor agonists 17 $\beta$ -estradiol and diethylstilbesterol. A different peptide was shown to distinguish between clomiphene bound to ER $\alpha$  and ER $\beta$ . These data indicate that each ligand potentially places the receptor in a unique and unpredictable conformation that is likely to have distinct biological activities.

As mentioned above, estrogens affect a panoply of biological processes. In addition, where gender differences have been described (e.g. disease frequencies, responses to challenge, etc), it is possible that the explanation involves the difference in estrogen levels between males and females.



## DESCRIPTION OF THE INVENTION

This invention provides estrogenic compound of formula I having the structure,



wherein

$R_1$  is hydrogen, hydroxyl, halogen, alkyl of 1-6 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, cycloalkyl of 3-8 carbon atoms, alkoxy of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, thioalkyl of 1-6 carbon atoms, sulfoxoalkyl of 1-6 carbon atoms, sulfonoalkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms, a 5 or 6-membered heterocyclic ring having 1 to 4 heteroatoms selected from O, N or S,  $-NO_2$ ,  $-NR_5R_6$ ,  $-N(R_5)COR_6$ ,  $-CN$ ,  $-CHFCN$ ,  $-CF_2CN$ , alkynyl of 2-7 carbon atoms, or alkenyl of 2-7 carbon atoms; wherein the alkyl or alkenyl moieties are optionally substituted with hydroxyl,  $-CN$ , halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms,  $-COR_5$ ,  $-CO_2R_5$ ,  $-NO_2$ ,  $CONR_5R_6$ ,  $NR_5R_6$  or  $N(R_5)COR_6$ ;

$R_2$  and  $R_{2a}$  are each, independently, hydrogen, hydroxyl, halogen, alkyl of 1-6 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of 2-7 carbon atoms, or alkynyl of 2-7 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl or alkenyl moieties are optionally substituted with hydroxyl,  $-CN$ , halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms,  $-COR_5$ ,  $-CO_2R_5$ ,  $-NO_2$ ,  $CONR_5R_6$ ,  $NR_5R_6$  or  $N(R_5)COR_6$ ;

$R_3$ ,  $R_{3a}$ , and  $R_4$  are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, halogen, alkoxy of 1-4 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl or alkenyl moieties are optionally substituted with hydroxyl,  $-CN$ , halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms,  $-COR_5$ ,  $-CO_2R_5$ ,  $-NO_2$ ,  $CONR_5R_6$ ,  $NR_5R_6$  or  $N(R_5)COR_6$ ;

R<sub>5</sub>, R<sub>6</sub> are each, independently hydrogen, alkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms;

X is O, S, or NR<sub>7</sub>;

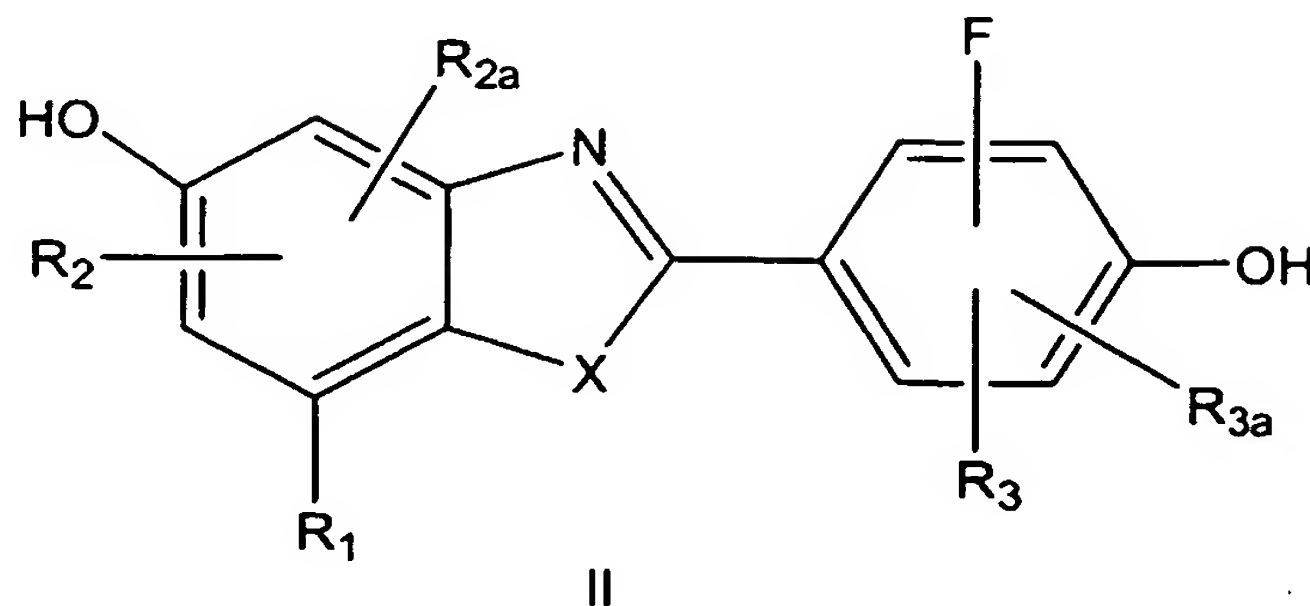
R<sub>7</sub> is hydrogen, alkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub> or -SO<sub>2</sub>R<sub>5</sub>;

or a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable salts can be formed from organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable acids when a compound of this invention contains a basic moiety. Salts may also be formed from organic and inorganic bases, such as alkali metal salts (for example, sodium, lithium, or potassium) alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, and trialkylammonium salts containing 1-6 carbon atoms in each alkyl group, when a compound of this invention contains an acidic moiety.

The terms alkyl, alkenyl, and alkynyl include both branched and straight chain moieties. Examples include methyl, ethyl, propyl, butyl, isopropyl, sec-butyl, tert-butyl, vinyl, allyl, acetylene, 1-methyl vinyl, and the like. When alkyl or alkenyl moieties are substituted, they may typically be mono-, di-, tri- or per-substituted. Examples for a halogen substituent include 1-bromo vinyl, 1-fluoro vinyl, 1,2-difluoro vinyl, 2,2-difluorovinyl, 1,2,2-trifluorovinyl, 1,2-dibromo ethane, 1,2 difluoro ethane, 1-fluoro-2-bromo ethane, CF<sub>2</sub>CF<sub>3</sub>, CF<sub>2</sub>CF<sub>2</sub>CF<sub>3</sub>, and the like. The term halogen includes bromine, chlorine, fluorine, and iodine. The term aryl means phenyl, 1-naphthyl, or 2-naphthyl. Preferred 5-6 membered heterocyclic rings include furan, thiophene, pyrrole, isopyrrole, pyrazole, imidazole, triazole, dithiole, oxathiole, isoxazole, oxazole, thiazole, isothiazole, oxadiazole, furazan, oxatriazole, dioxazole, oxathiazole, tetrazole, pyran, pyridine, pyridazine, pyrimidine, pyrazine, triazine, oxazine, oxathiazine, or oxadiazine. It is more preferred that the heterocyclic ring is furan, thiophene, or thiazole.

Of the compounds of this invention, it is preferred that the compound of formula I has the structure



5

wherein

$R_1$  is alkenyl of 2-7 carbon atoms; wherein the alkenyl moiety is optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;

10  $R_2$  and  $R_{2a}$  are each, independently, hydrogen, hydroxyl, halogen, alkyl of 1-6 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl, alkenyl, or alkynyl moieties are optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;

15

$R_3$ , and  $R_{3a}$  are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, halogen, alkoxy of 1-4 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl, alkenyl, or alkynyl moieties are optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;

20

$R_5$ ,  $R_6$  are each, independently hydrogen, alkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms;

25 X is O, S, or NR<sub>7</sub>;

$R_7$  is hydrogen, alkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub> or -SO<sub>2</sub>R<sub>5</sub>;

or a pharmaceutically acceptable salt thereof.

It is more preferred that X is O, and still more preferred that X is O, and R<sub>1</sub> is alkenyl of 2-3 carbon atoms, which is optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>.

As used in accordance with this invention, the term "providing," with respect to providing a compound or substance covered by this invention, means either directly administering such a compound or substance, or administering a prodrug, derivative, or analog which will form the effective amount of the compound or substance within the body.

As used in accordance with this invention, the term "ER $\beta$  selective ligand" means that the binding affinity (as measured by IC<sub>50</sub>, where the IC<sub>50</sub> of 17 $\beta$ -estradiol is not more than 3 fold different between ER $\alpha$  and ER $\beta$ ) of the ligand to ER $\beta$  is at least about 10 times greater than its binding affinity to ER $\alpha$  in a standard pharmacological test procedure that measures the binding affinities to ER $\alpha$  and ER $\beta$ . It is preferred that the ER $\beta$  selective ligand will have a binding affinity to ER $\beta$  that is at least about 20 times greater than its binding affinity to ER $\alpha$ . It is more preferred that the ER $\beta$  selective ligand will have a binding affinity to ER $\beta$  that is at least about 50 times greater than its binding affinity to ER $\alpha$ . It is further preferred that the ER $\beta$  selective ligand is non-uterotrophic and non-mammatrophic.

As used in accordance with this invention, the term "non-uterotrophic" means producing an increase in wet uterine weight in a standard pharmacological test procedure of less than about 50% of the uterine weight increase observed for a maximally efficacious dose of 17 $\beta$ -estradiol or 17 $\alpha$ -ethinyl-17 $\beta$ -estradiol in the same procedure. It is preferred that the increase in wet uterine weight will be less than about 25% of that observed for estradiol, and more preferred that the increase in wet uterine weight will be less than about 10% of that observed for estradiol. It is most preferred that the non-uterotrophic ER $\beta$  selective ligand will not increase wet uterine

weight significantly ( $p > 0.05$ ) compared with a control that is devoid of uterotrophic activity (e.g. vehicle).

As used in accordance with this invention, the term "non-mammotrophic" means producing an increase in casein kinase II mRNA in a standard pharmacological test procedure of less than about 50% of the casein kinase II mRNA increase observed for a maximally efficacious dose of  $17\beta$ -estradiol or  $17\alpha$ -ethinyl- $17\beta$ -estradiol in the same procedure. It is preferred that the increase casein kinase II mRNA will be less than about 25% of that observed for estradiol, and more preferred that the increase in casein kinase II mRNA will be less than about 10% of that observed for estradiol. It is most preferred that the non-mammotrophic  $ER\beta$  selective ligand will not increase casein kinase II mRNA significantly ( $p > 0.05$ ) compared with a control that is devoid of mammotrophic activity (e.g. vehicle).

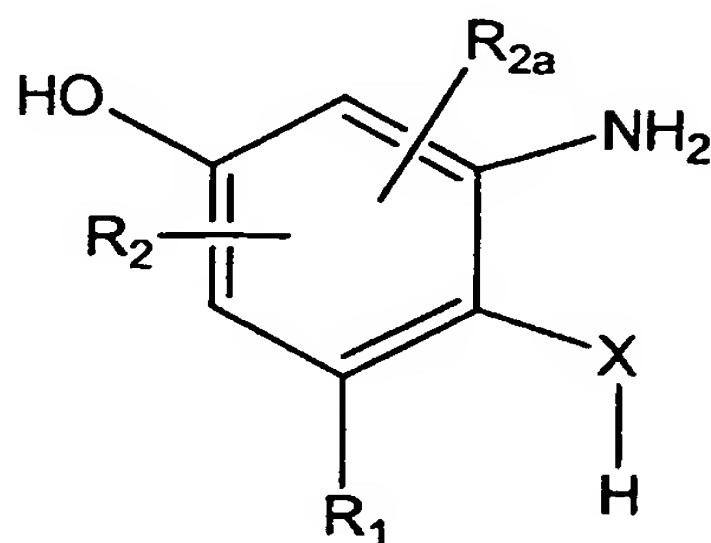
This invention also provides the use of a  $ER\beta$  selective ligand in the treatment or inhibition of arthritis, inflammatory bowel disease, and endometriosis. More particularly, the  $ER\beta$  selective ligands are useful in the treatment or inhibition of rheumatoid arthritis, osteoarthritis or spondyloarthropathies; and Crohn's disease, ulcerative colitis, indeterminate colitis, infectious colitis, or ulcerative proctitis. This invention further provides for the use of an  $ER\beta$  selective ligand in treating or inhibiting joint swelling or erosion; or treating or inhibiting joint damage secondary to arthroscopic or surgical procedures. It is preferred that the  $ER\beta$  selective ligand is non-uterotrophic and non-mammotrophic.

The reagents used in the preparation of the compounds of this invention can be either commercially obtained or can be prepared by standard procedures described in the literature.

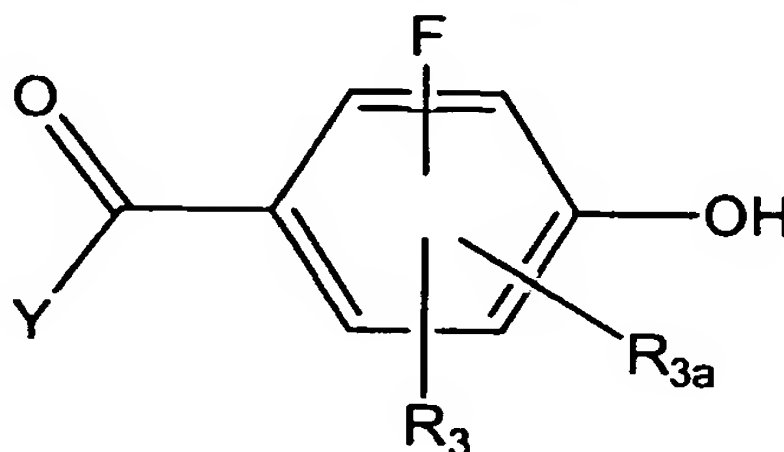


In another aspect, this invention relates to a process for the preparation of a compound of the invention comprising one of the following:

- a) reacting a compound of formula



wherein  $R_1$ ,  $R_2$ ,  $R_{2a}$  and  $X$  are as defined above with a compound of formula



wherein  $R_3$  and  $R_{3a}$  are as defined above and  $Y$  is halogen,  $-OH$  or alkoxy of 1 to 6 carbon atoms;

or

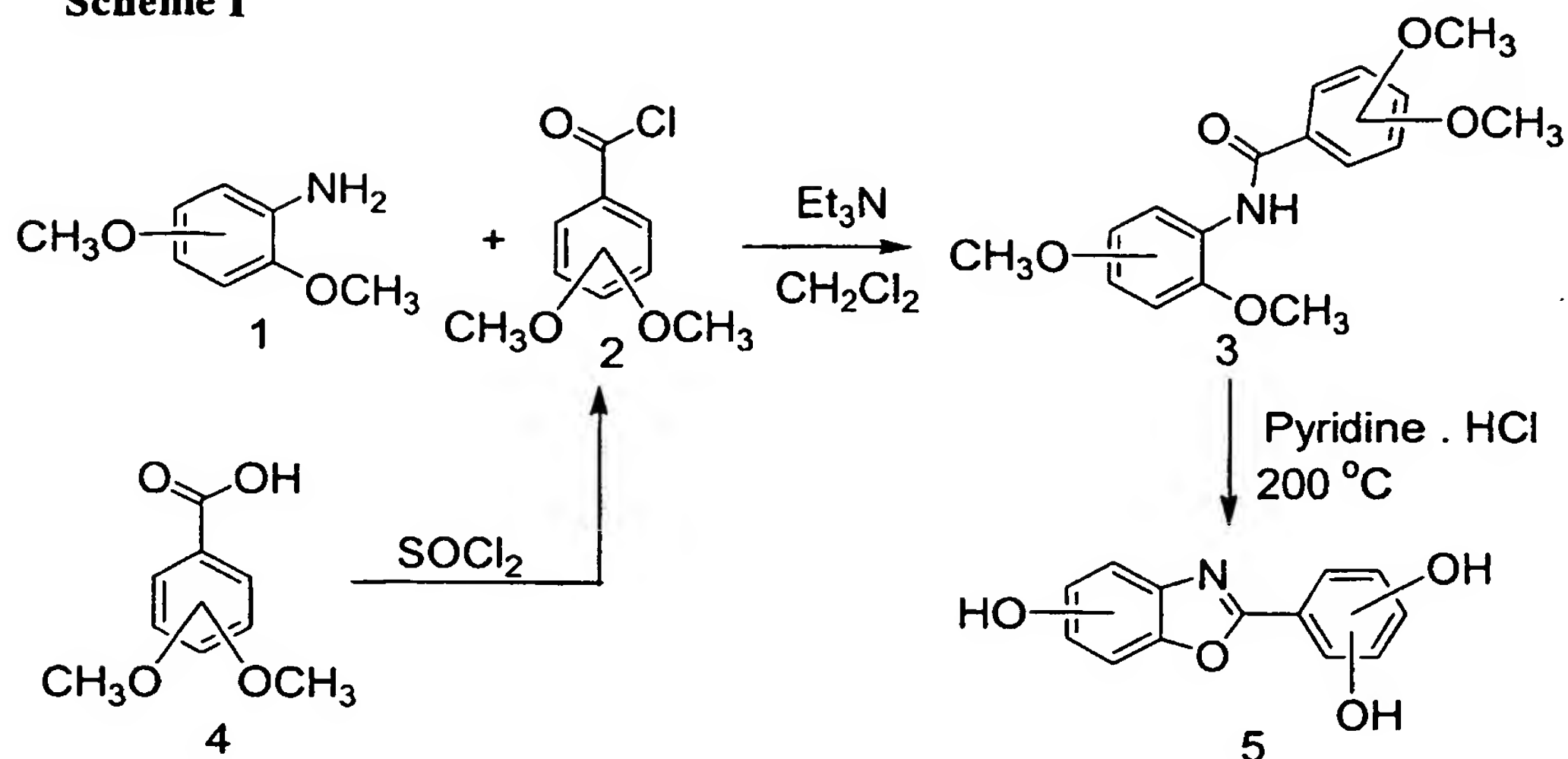
- b) converting a compound of formula II as defined above to a pharmaceutically acceptable salt thereof;

or

- c) resolving an isomeric mixture of compounds of formula II to isolate an enantiomer of a compound of formula II or a pharmaceutically acceptable salt thereof.

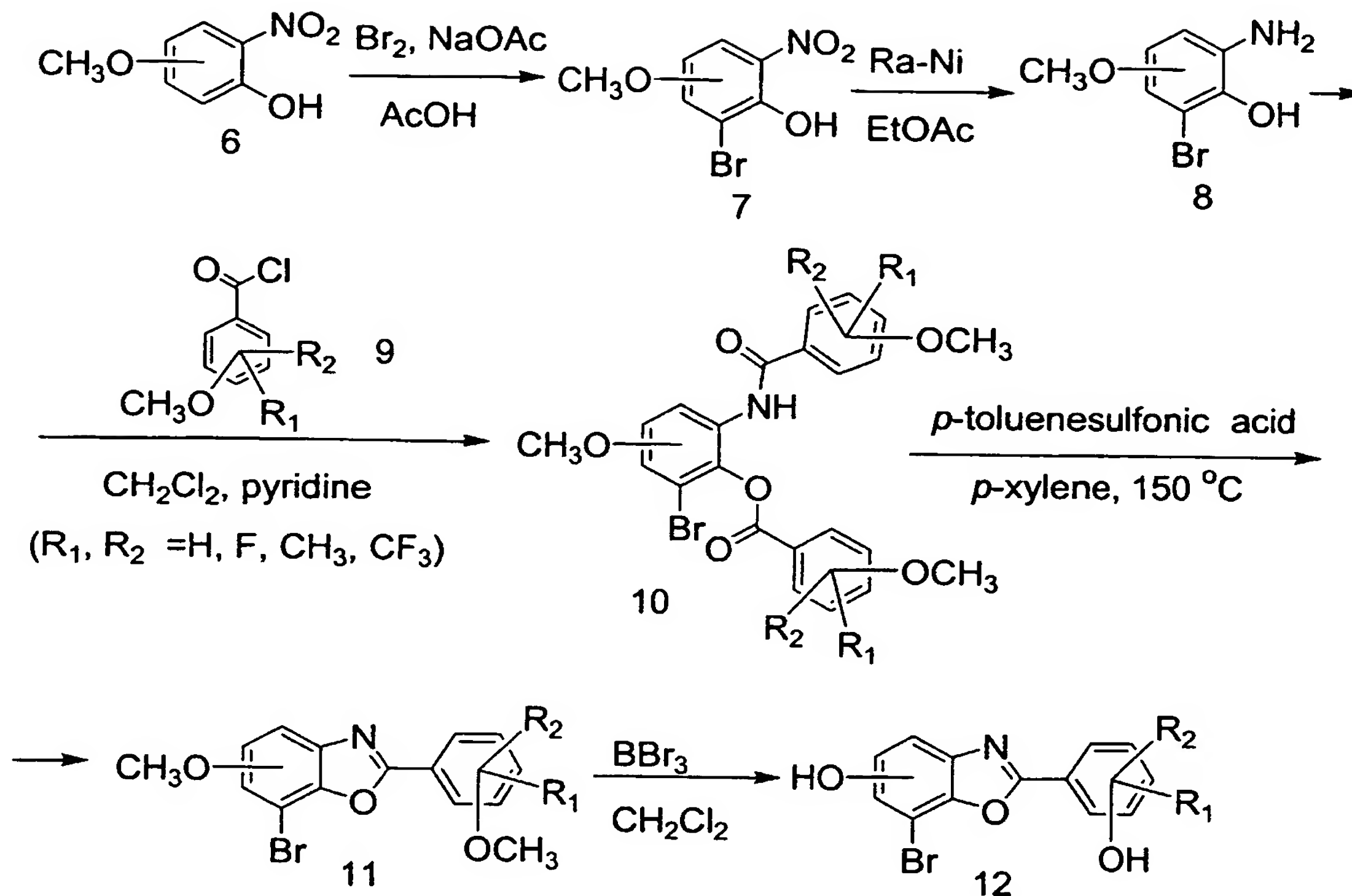
The compounds of the present invention may for example be prepared according to the following synthetic Schemes (I-VIII)

Scheme I



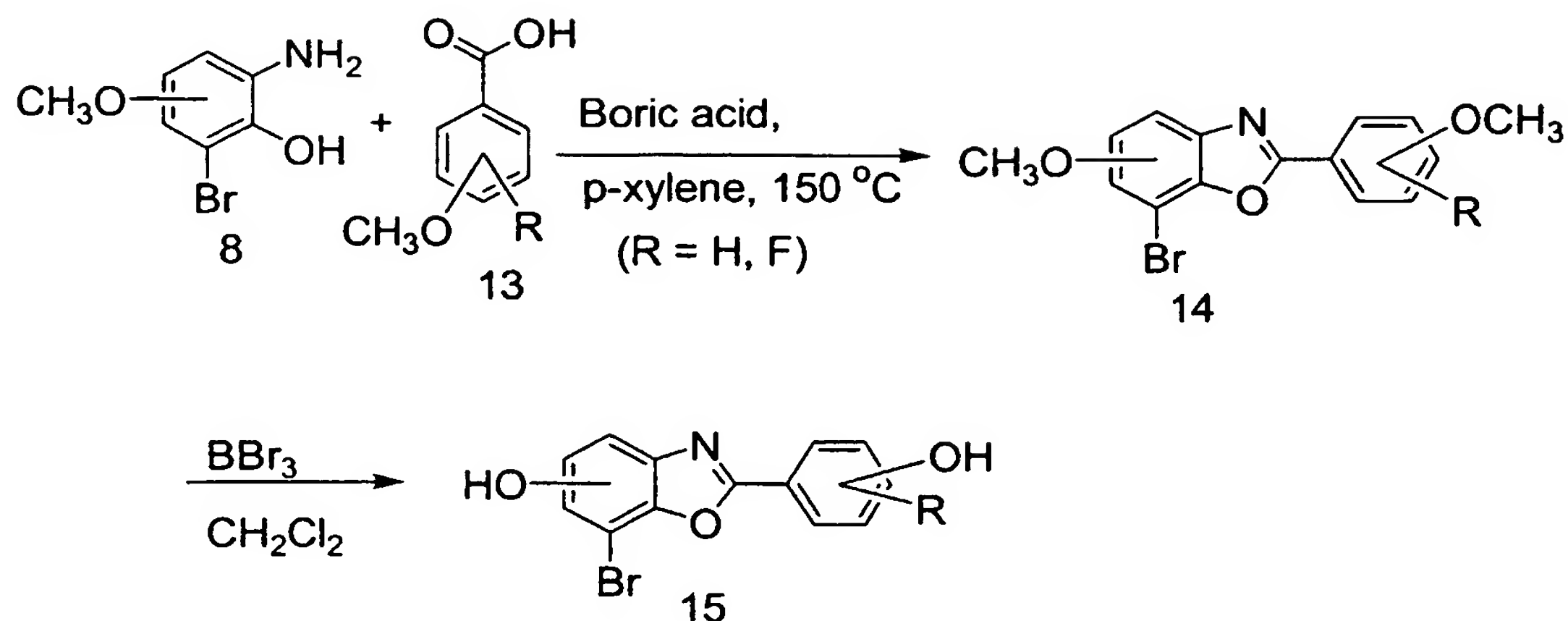
In Scheme I, commercially available dimethoxy aniline 1 was treated with  
 5 commercially available benzoyl chloride 2 in the presence of triethylamine to produce  
 amide 3. The required benzoyl chloride 2 was also prepared from commercially  
 available benzoic acid 4 upon refluxing with thionyl chloride. Amide 3 was converted  
 to the phenolic benzoxazole 5 upon treatment with pyridine hydrochloride at high  
 temperature ( $200^\circ\text{C}$ ).

## Scheme II



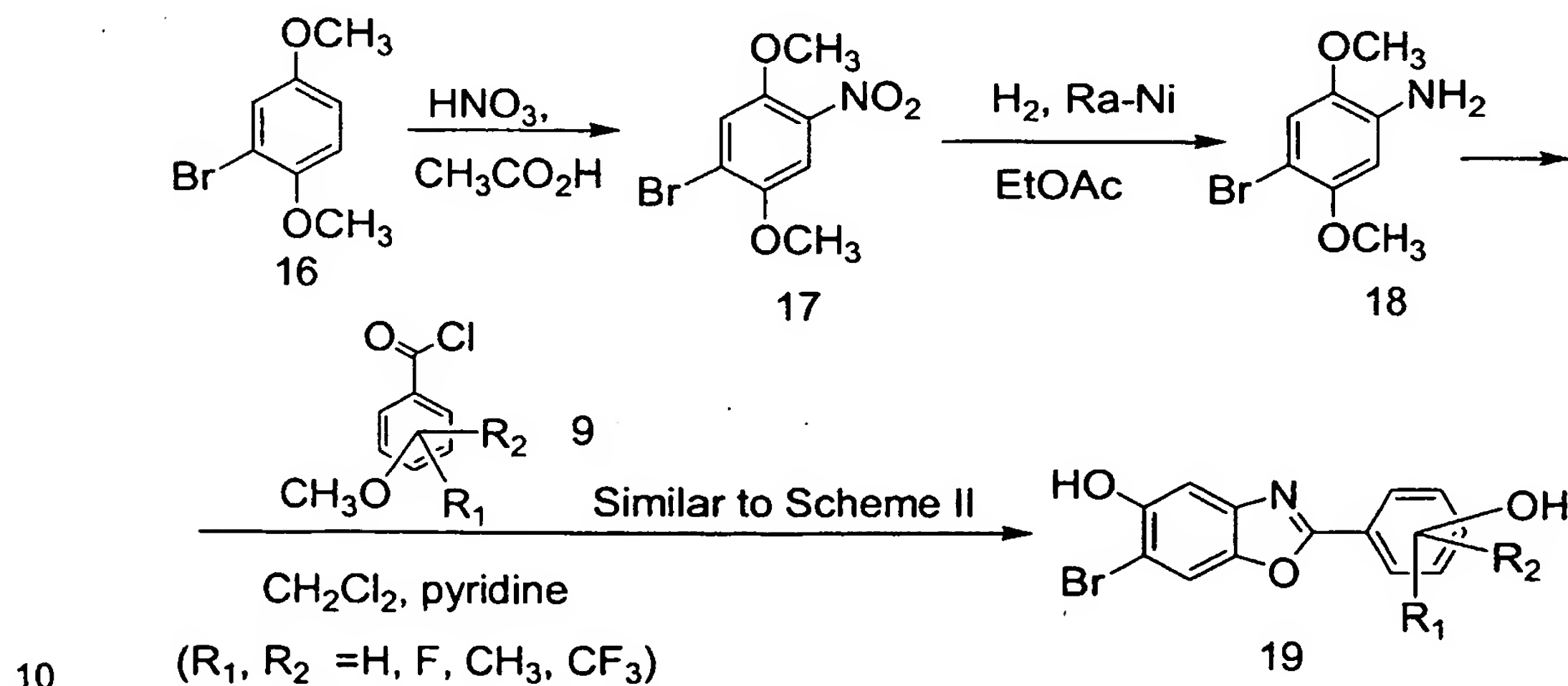
- 5 In Scheme II, commercially available nitro-phenol 6 was brominated with  $\text{Br}_2/\text{NaOAc}$  in acetic acid to produce bromo-phenol 7. Catalytic hydrogenation of 7 with  $\text{Ra-Ni}$  in  $\text{EtOAc}$  afforded aniline 8. Coupling of 8 with benzoyl chloride 9 (commercially available, or prepared from the corresponding benzoic acid and thionyl chloride) in the presence of pyridine produced amide-ester 10. Conversion of 10 to benzoxazole 11 was accomplished under acidic conditions ( $p$ -toluenesulfonic acid) at high temperature ( $150^\circ\text{C}$ ). Demethylation of 11 with boron tribromide in dichloromethane afforded the phenolic benzoxazole 12.

## Scheme III



In Scheme III, aniline 8 was converted to benzoxazole 14 upon treatment with 5 benzoic acid 13 and boric acid in *p*-xylene at high temperature (150°C). Demethylation of 14 with boron tribromide in dichloromethane produced the phenolic benzoxazole 15.

## Scheme IV

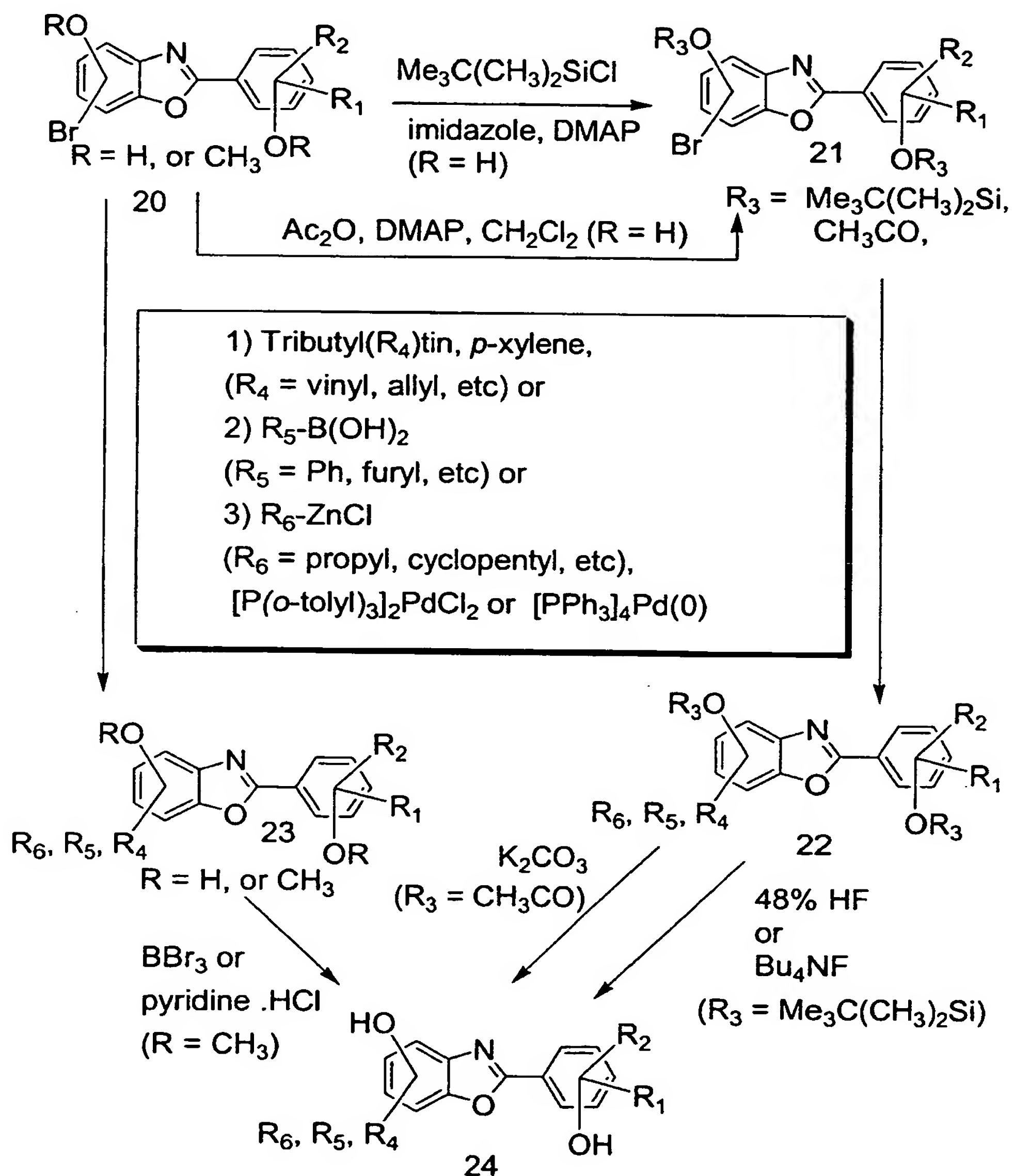


In Scheme IV, nitration of 16 with nitric acid in acetic acid produced 17, which was reduced with hydrogen in the presence of Ra-Ni to afford aniline 18. Aniline 18

was converted to benzoxazole 19 in a similar manner as described in Scheme II, with the exemption that the demethylation step was accomplished with pyridine hydrochloride at high temperature (200 °C).

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Scheme V

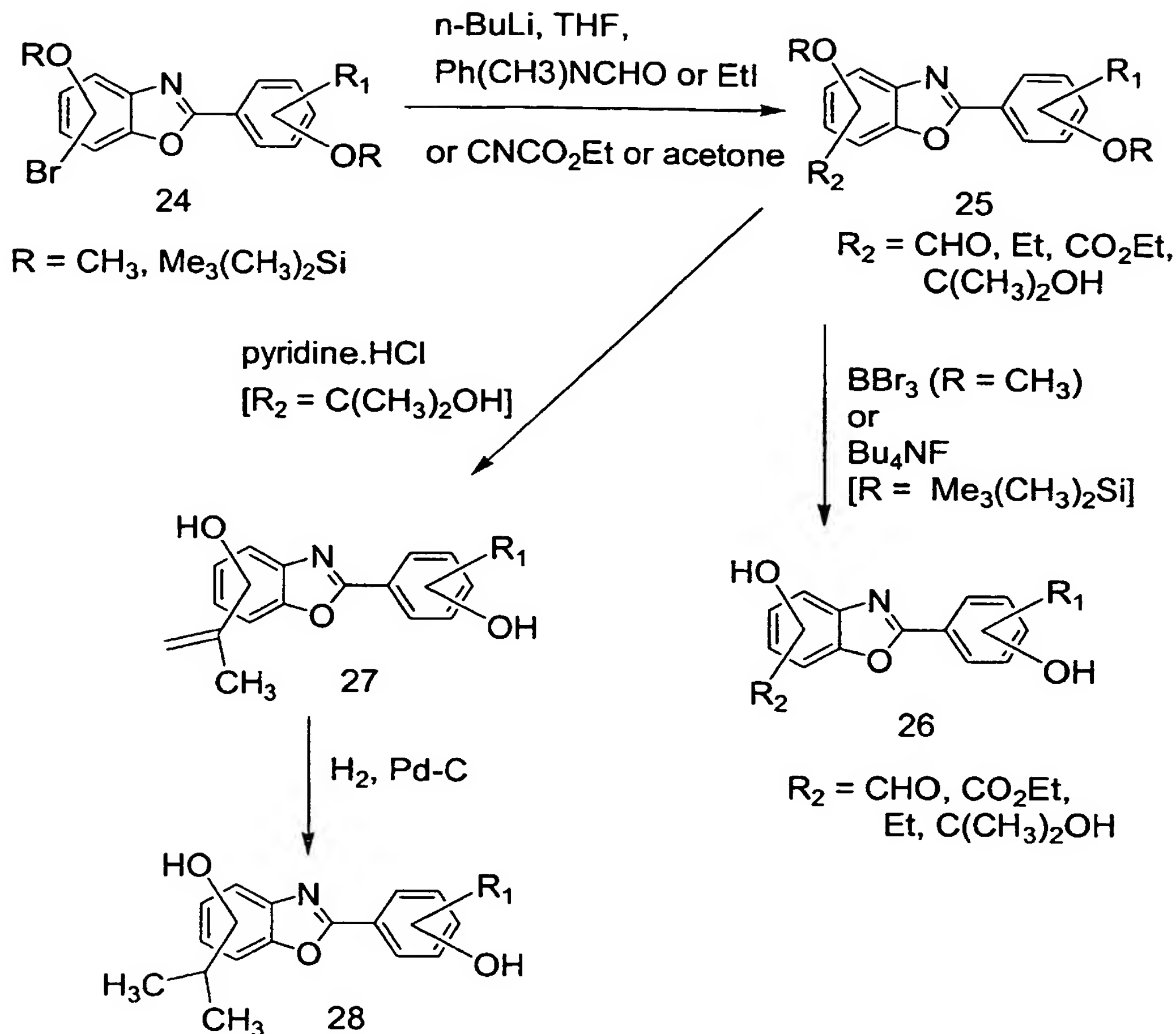




In Scheme V, the hydroxyl groups of benzoxazole 20 were protected either as the silyl ethers 21 ( $R_3 = \text{Me}_3\text{C}(\text{CH}_3)_2\text{Si}$ ) with tert-butyldimethylsilyl chloride/imidazole/4-dimethylaminopyridine in N,N-dimethylformamide, or as the esters 21 ( $R_3 = \text{CH}_3\text{CO}$ ) with acetic anhydride/4-dimethylaminopyridine in dichloromethane. Benzoxazoles 20 and 21 were coupled with a variety of tin reagents (i.e., tributyl(vinyl)tin, tributyl(allyl)tin, tributyl(2-furyl)tin, boronic acids or zinc chlorides in the presence of a palladium catalyst [i.e. dichlorobis(tri-*o*-tolylphosphine)palladium(II) or tetrakis(triphenylphosphine)palladium(0)] in *p*-xylene, toluene, tetrahydrofuran, dimethoxymethane or 1,2-dimethoxyethane, with the presence of a base (i.e.  $\text{Na}_2\text{CO}_3$ ) for the boronic acid coupling reaction, at temperatures in the range of 20 °C to 150 °C, to produce benzoxazoles 22 and 23.

Deprotection of the silyl ethers of 22 ( $R_3 = \text{Me}_3\text{C}(\text{CH}_3)_2\text{Si}$ ) with hydrofluoric acid (48 wt.% in water) or tetrabutylammonium fluoride produced benzoxazole 24. Saponification of 22 ( $R_3 = \text{CH}_3\text{CO}$ ) with potassium carbonate in dioxane produced benzoxazole 24. Benzoxazole 23 ( $R = \text{CH}_3$ ) was demethylated with boron tribromide in dichloromethane or pyridine hydrochloride at high temperature (200 °C) to afford benzoxazole 24.

## Scheme VI

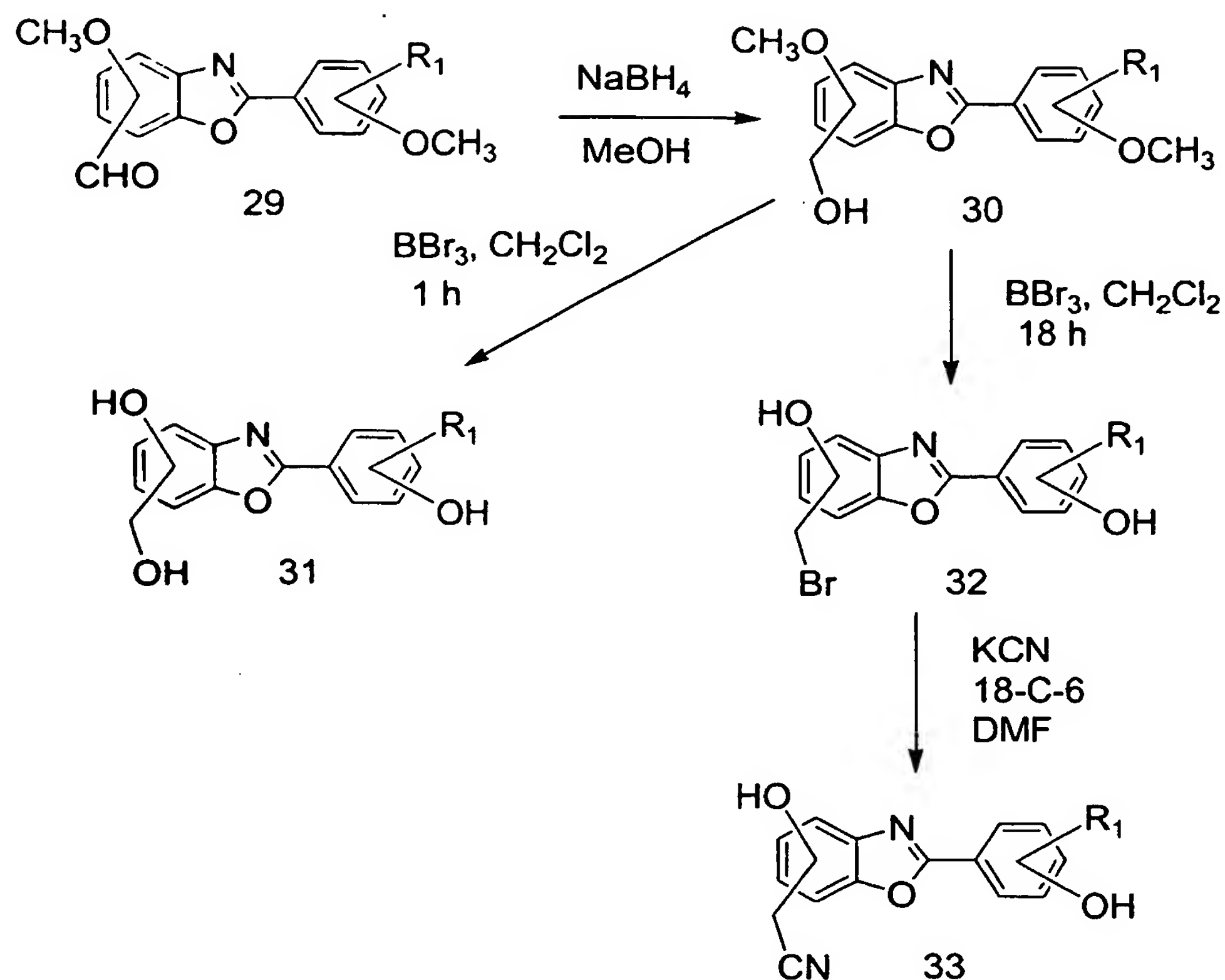


In Scheme VI, benzoxazole 24 was treated with n-butyllithium at low temperatures ( $-78^\circ C$ ) followed by the addition of an electrophile (i.e.  $CNCO_2Et$ ,  $Ph(CH_3)NCHO$ ,  $EtI$ , etc) to produce compound 25. Deprotection of 25 with boron tribromide ( $R = CH_3$ ) or tetrabutylammonium fluoride ( $R = Me_3C(CH_3)_2Si$ ) afforded benzoxazole 26 [ $R = CHO, CO_2Et, CH_2CH_3, C(CH_3)_2OH$ ].

The tertiary alcohol 25 ( $R = C(CH_3)_2OH$ ) was treated with pyridine hydrochloride at high temperature ( $200^\circ C$ ) to produce 1-methyl-vinyl benzoxazole 27.

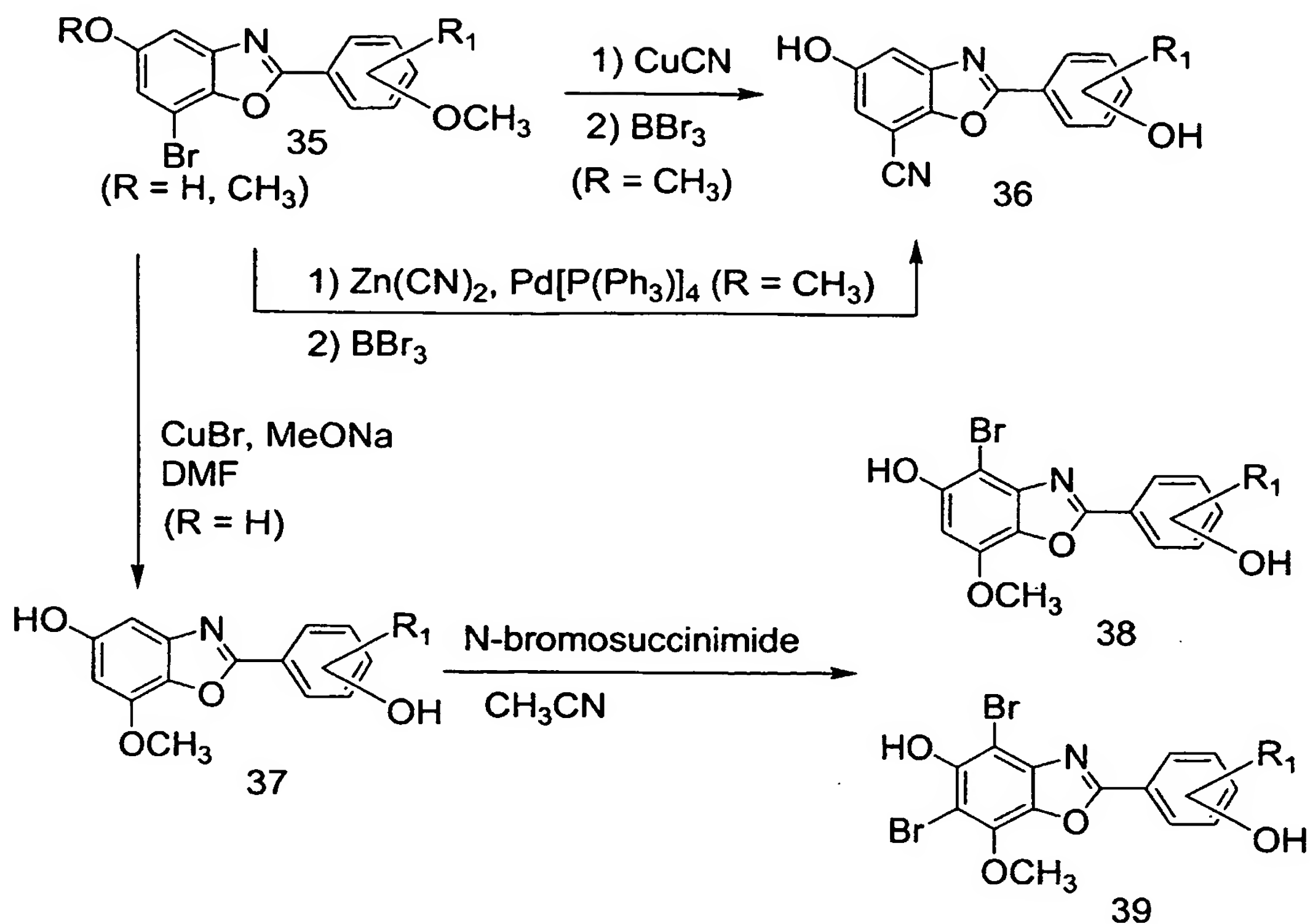
Reduction of 27 with  $H_2$  /  $Pd-C$  afforded the isopropyl analog 28.

## Scheme VII



In Scheme VII, reduction of the benzoxazole 29 with sodium borohydride in methanol produced alcohol 30. Treatment of 30 with boron tribromide in  $\text{CH}_2\text{Cl}_2$  for 1  
5 hour furnished benzoxazole 31, while prolonged (18 hours) treatment afforded bromide 32. Bromide 32 was converted to acetonitrile 33 upon treatment with potassium cyanide and 18-crown-6 ether in N,N-dimethylformamide.

## Scheme VIII



- In Scheme VIII, bromo-benzoxazole 35 ( $R = \text{CH}_3$ ) was first treated with copper(I) cyanide in DMF to produce the corresponding aryl-nitrile, which upon treatment with boron tribromide afforded benzoxazole 36. Benzoxazole 36 was also prepared from a second synthetic Route, where the bromo-benzoxazole 35 was treated with zinc cyanide in the presence of a palladium catalyst [i.e. tetrakis(triphenylphosphine)palladium(0)] to afford the corresponding aryl-nitrile, which upon demethylation with boron tribromide produced benzoxazole 36.
- Benzoxazole 35 ( $R = \text{H}$ ) was treated with copper (I) bromide, and freshly prepared sodium methoxide in DMF to produce methoxy-benzoxazole 37. Bromination of 37 with N-bromosuccinimide in acetonitrile afforded the monobromo benzoxazole 38 (major product) and the dibromobenzoxazole 39 (minor product).

Standard pharmacological test procedures are readily available to determine the activity profile of a given test compound. The following briefly summarizes several representative test procedures and may include data for representative compounds of the invention. All assays, except the radioligand binding assay, can be used to detect estrogen receptor agonist or antagonist activity of compounds. In general, estrogen receptor agonist activity is measured by comparing the activity of the compound to a reference estrogen (e.g.  $17\beta$ -estradiol,  $17\alpha$ -ethinyl,  $17\beta$ -estradiol, estrone, diethylstilbesterol etc). Estrogen receptor antagonist activity is generally measured by co-treating the test compound with the reference estrogen and comparing the result to that obtained with the reference estrogen alone. Standard pharmacological test procedures for SERMs are also provided in US Patents 4,418,068 and 5,998,402 which are hereby incorporated by reference.

#### Evaluation of binding affinities to ER $\alpha$ and ER $\beta$

Representative examples of the invention were evaluated for their ability to compete with  $17\beta$ -estradiol for both ER $\alpha$  and ER $\beta$  in a conventional radioligand binding assay. This test procedure provides the methodology for one to determine the relative binding affinities for the ER $\alpha$  or ER $\beta$  receptors. The procedure used is briefly described below.

*Preparation of receptor extracts for characterization of binding selectivity.* The ligand binding domains, conveniently defined here as all sequence downstream of the DNA binding domain, were obtained by PCR using full length cDNA as templates and primers that contained appropriate restriction sites for subcloning while maintaining the appropriate reading frame for expression. These templates contained amino acids M<sub>250</sub>-V<sub>595</sub> of human ER $\alpha$  [Green, et al., Nature 320: 134-9 (1986)] and M<sub>214</sub>-Q<sub>530</sub> of human ER $\beta$  [Ogawa, et al., Biochemical & Biophysical Research Communications 243: 122-6 (1998)]. Human ER $\beta$  was cloned into pET15b (Novagen, Madison WI) as a Nco1-BamH1 fragment bearing a C-terminal Flag tag. Human ER $\alpha$  was cloned as for human ER $\beta$  except that an N-terminal His tag was added. The sequences of all constructs used were verified by complete sequencing of both strands.

BL21(DE3) cells were used to express the human proteins. Typically a 10 mL overnight culture was used to inoculate a 1 L culture of LB medium containing 100  $\mu$ g/mL of ampicillin. After incubation overnight at 37 °C, IPTG was added to a final



concentration of 1 mM and incubation proceeded at 25°C for 2 hours. Cells were harvested by centrifugation (1500 x g) , and the pellets washed with and resuspended in 100 mL of 50 mM Tris-Cl (pH 7.4), 150 mM NaCl. Cells were lysed by passing twice through a French press at 12000 psi. The lysate was clarified by centrifugation at 12,000 x g for 30 minutes at 4 °C and stored at -70°C.

*Evaluation of extracts for specific [<sup>3</sup>H]-estradiol binding.* Dulbecco's phosphate buffered saline (Gibco, 1x final concentration) supplemented with 1 mM EDTA was used as the assay buffer. To optimize the amount of receptor to use in the assay, [<sup>3</sup>H]-17β-estradiol (New England Nuclear; final concentration = 2 nM) ± 0.6 μM diethylstilbestrol and 100 μL of various dilutions of the E. coli lysate were added to each well of a high binding masked microtiter plate (EG&G Wallac). The final assay volume was 120 μL and the concentration of DMSO was ≤ 1%. After incubation at room temperature for 5-18 hours, unbound material was aspirated and the plate washed three times with approximately 300 μL of assay buffer. After washing, 135 μL of scintillation cocktail (Optiphase Supermix, EG&G Wallac) was added to the wells, and the plate was sealed and agitated for at least 5 minutes to mix scintillant with residual wash buffer. Bound radioactivity was evaluated by liquid scintillation counting (EG&G Wallac Microbeta Plus).

After determining the dilution of each receptor preparation that provided maximum specific binding, the assay was further optimized by estimating the IC<sub>50</sub> of unlabelled 17β-estradiol using various dilutions of the receptor preparation. A final working dilution for each receptor preparation was chosen for which the IC<sub>50</sub> of unlabelled 17β-estradiol was 2-4 nM.

*Ligand binding competition test procedure.* Test compounds were initially solubilized in DMSO and the final concentration of DMSO in the binding assay was ≤1%. Eight dilutions of each test compound were used as an unlabelled competitor for [<sup>3</sup>H]-17β-estradiol. Typically, a set of compound dilutions would be tested simultaneously on human ERα and ERβ. The results were plotted as measured DPM vs. concentration of test compound. For dose-response curve fitting, a four parameter logistic model on the transformed, weighted data was fit and the IC<sub>50</sub> was defined as the concentration of compound decreasing maximum [<sup>3</sup>H]-estradiol binding by 50%.

Binding affinities for ERα and ERβ (as measured by IC<sub>50</sub>) for representative examples of the invention are shown in Table (1).

| Table 1: ER binding affinities of representative compounds of the invention |                                   |                                    |
|---|-----------------------------------|------------------------------------|
| Example   | ER- $\beta$ IC <sub>50</sub> (nM) | ER- $\alpha$ IC <sub>50</sub> (nM) |
| 1   | 140                               | 720                                |
| 2   | 963                               | 5110                               |
| 3   | 66                                | 1570                               |
| 4   | 239                               | 5280                               |
| 5   | 59                                | 139                                |
| 6   | 39                                | 843                                |
| 7   | 1600                              | 5000                               |
| 8   | 181                               | 2353                               |
| 9   | 440                               | 1500                               |
| 10  | 105                               | 2040                               |
| 11  | 703                               | >5000                              |
| 12  | 49                                | 1227                               |
| 13  | 25                                | 190                                |
| 14  | 50                                | 902                                |
| 15  | 3                                 | 82                                 |
| 16  | 64                                | 1813                               |
| 17  | 42                                | 1210                               |
| 18  | 16                                | 464                                |
| 19  | 157                               | 2765                               |
| 20  | 2                                 | 155                                |
| 21  | 3                                 | 260                                |
| 22  | 1                                 | 47                                 |
| 23  | 3                                 | 113                                |
| 24  | 6                                 | 1217                               |
| 25  | 2                                 | 227                                |
| 26  | 4                                 | 474                                |
| 27  | 4                                 | 409                                |
| 28  | 25                                | 1036                               |
| 29  | 155                               | 803                                |
| 30  | 134                               | 3080                               |
| 31  | 31                                | 352                                |
| 32  | 16                                | 196                                |
| 33  | 31                                | 352                                |
| 34  | 14                                | 1101                               |
| 35  | 15                                | 481                                |
| 36  | 11                                | 390                                |
| 37  | 79                                | 498                                |
| 38  | 102                               | 1010                               |
| 39  | 190                               | 7827                               |
| 40  | 235                               | 1300                               |
| 41  | 6                                 | 411                                |
| 42  | 95                                | 9620                               |
| 43  | 59                                | 2557                               |
| 44  | 13                                | 537                                |
| 45  | 84                                | 655                                |
| 46  | 59                                | 2638                               |
| 47  | 1340                              | Not determined                     |
| 48  | 40                                | 2975                               |

| Table 1: ER binding affinities of representative compounds of the invention |      |        |
|---|------|--------|
| 49  | 1042 | 5230   |
| 50  | 399  | >5000  |
| 51  | 142  | 775    |
| 52  | 82   | 1200   |
| 53  | 166  | 1870   |
| 54  | 135  | 809    |
| 55  | 313  | 1980   |
| 56  | 97   | 1030   |
| 57  | 366  | 1340   |
| 58  | 26   | 1435   |
| 59  | 52   | 2668   |
| 60  | 64   | 559    |
| 61  | 93   | 1180   |
| 62  | 201  | >10000 |
| 63  | 1    | 44     |
| 64  | 3    | 376    |

The results obtained in the standard pharmacologic test procedure described  
 5 above demonstrate that the compounds of this invention bind both subtypes of the  
 estrogen receptor. The  $IC_{50}$ s are generally lower for  $ER\beta$ , indicating these  
 compounds are preferentially  $ER\beta$  selective ligands, but are still considered active at  
 $ER\alpha$ . Compounds of this invention will exhibit a range of activity based, at least  
 partially, on their receptor affinity selectivity profiles. Since the compounds of the  
 10 invention bind  $ER\beta$  with higher affinity than  $ER\alpha$ , they will be useful in treating or  
 inhibiting diseases than can be modulated via  $ER\beta$ . Additionally, since each receptor  
 ligand complex is unique and thus its interaction with various coregulatory proteins is  
 unique, compounds of this invention will display different and unpredictable activities  
 depending on cellular context. For example, in some cell-types, it is possible for a  
 15 compound to behave as an estrogen receptor agonist while in other tissues, an  
 estrogen receptor antagonist. Compounds with such activity have sometimes been  
 referred to as SERMs (Selective Estrogen Receptor Modulators). Unlike many  
 estrogens, however, many of the SERMs do not cause increases in uterine wet  
 weight. These compounds are antiestrogenic in the uterus and can completely  
 20 antagonize the trophic effects of estrogen receptor agonists in uterine tissue. These  
 compounds, however, act as estrogen receptor agonists in the bone, cardiovascular,  
 and central nervous systems. Due to this tissue selective nature of these compounds,

they are useful in treating or inhibiting in a mammal disease states or syndromes which are caused or associated with an estrogen deficiency (in certain tissues such as bone or cardiovascular) or an excess of estrogen (in the uterus or mammary glands). In addition, compounds of this invention also have the potential to behave as estrogen  
5 receptor agonists on one receptor type while behaving as estrogen receptor antagonists on the other. For example, it has been demonstrated that compounds can be antagonize the action of  $17\beta$ -estradiol via  $ER\beta$  while exhibiting estrogen receptor agonist activity with  $ER\alpha$  [Sun, et al., Endocrinology 140: 800-804 (1999)]. Such ERSAA (Estrogen Receptor Selective Agonist Antagonist) activity provides for  
10 pharmacologically distinct estrogenic activity within this series of compounds

#### Regulation of metallothionein II mRNA

Estrogens acting through  $ER\beta$ , but not  $ER\alpha$  can upregulate metallothionein II mRNA levels in Saos-2 cells as described by Harris [Endocrinology 142: 645-652  
15 (2001)]. Results from this test procedure can be combined with results from the test procedure described below (ERE reporter test procedure) to generate a selectivity profile for compounds of this invention (see also WO 00/37681). Data for representative compounds of the invention are shown in Table (2).

| Table 2: Regulation of metallothionein-II mRNA in Saos-2 cells |                 |
|--|-----------------|
| Compound   | Fold regulation |
| Example 12   | 9.6             |
| Example 14   | 12.4            |
| Example 13   | 9.7             |

20

#### Evaluation of test compound using an ERE-reporter test procedure in MCF-7 breast cancer cells

Stock solutions of test compounds (usually 0.1 M) are prepared in DMSO and then diluted 10 to 100-fold with DMSO to make working solutions of 1 or 10 mM. The  
25 DMSO stocks are stored at either  $4^{\circ}\text{C}$  (0.1 M) or  $-20^{\circ}\text{C}$  ( $< 0.1\text{M}$ ). MCF-7 cells are passaged twice a week with growth medium [D-MEM/F-12 medium containing 10% (v/v) heat-inactivated fetal bovine serum, 1% (v/v) Penicillin-Streptomycin, and 2 mM glutaMax-1]. The cells are maintained in vented flasks at  $37^{\circ}\text{C}$  inside a 5%  $\text{CO}_2/95\%$

humidified air incubator. One day prior to treatment, the cells are plated with growth medium at 25,000 cells/well into 96 well plates and incubated at 37 °C overnight.

The cells are infected for 2 hr at 37 °C with 50 µl/well of a 1:10 dilution of adenovirus 5-ERE-tk-luciferase in experimental medium [phenol red-free D-MEM/F-12 medium containing 10% (v/v) heat-inactivated charcoal-stripped fetal bovine serum, 1% (v/v) Penicillin-Streptomycin, 2 mM glutaMax-1, 1 mM sodium pyruvate]. The wells are then washed once with 150 µl of experimental medium. Finally, the cells are treated for 24 hr at 37 °C in replicates of 8 wells/treatment with 150 µl/well of vehicle ( $\leq 0.1\%$  v/v DMSO) or compound that is diluted  $\geq 1000$ -fold into experimental medium.

Initial screening of test compounds is done at a single dose of 1 µM that is tested alone (estrogen receptor agonist mode) or in combination with 0.1 nM 17β-estradiol (EC<sub>80</sub>; estrogen receptor antagonist mode). Each 96 well plate also includes a vehicle control group (0.1% v/v DMSO) and an estrogen receptor agonist control group (either 0.1 or 1 nM 17β-estradiol). Dose-response experiments are performed in either the estrogen receptor agonist and/or estrogen receptor antagonist modes on active compounds in log increases from 10<sup>-14</sup> to 10<sup>-5</sup> M. From these dose-response curves, EC<sub>50</sub> and IC<sub>50</sub> values, respectively, are generated. The final well in each treatment group contains 5 µl of 3 x 10<sup>-5</sup> M ICI-182,780 (10<sup>-6</sup> M final concentration) as an estrogen receptor antagonist control.

After treatment, the cells are lysed on a shaker for 15 min with 25 µl/well of 1X cell culture lysis reagent (Promega Corporation). The cell lysates (20 µl) are transferred to a 96 well luminometer plate, and luciferase activity is measured in a MicroLumat LB 96 P luminometer (EG & G Berthold) using 100 µl/well of luciferase substrate (Promega Corporation). Prior to the injection of substrate, a 1 second background measurement is made for each well. Following the injection of substrate, luciferase activity is measured for 10 seconds after a 1 second delay. The data are transferred from the luminometer to a Macintosh personal computer and analyzed using the JMP software (SAS Institute); this program subtracts the background reading from the luciferase measurement for each well and then determines the mean and standard deviation of each treatment.

The luciferase data are transformed by logarithms, and the Huber M-estimator is used to down-weight the outlying transformed observations. The JMP software is



used to analyze the transformed and weighted data for one-way ANOVA (Dunnett's test). The compound treatments are compared to the vehicle control results in the estrogen receptor agonist mode, or the positive estrogen receptor agonist control results (0.1 nM 17 $\beta$ -estradiol) in the estrogen receptor antagonist mode. For the initial single dose experiment, if the compound treatment results are significantly different from the appropriate control ( $p < 0.05$ ), then the results are reported as the percent relative to the 17 $\beta$ -estradiol control [i.e., ((compound - vehicle control)/(17 $\beta$ -estradiol control - vehicle control)) x 100]. The JMP software is also used to determine the EC<sub>50</sub> and/or IC<sub>50</sub> values from the non-linear dose-response curves.

#### Evaluation of uterotrophic activity

Uterotrophic activity of a test compound can be measured according to the following standard pharmacological test procedures.

Procedure 1: Sexually immature (18 days of age) Sprague-Dawley rats are obtained from Taconic and provided unrestricted access to a casein-based diet (Purina Mills 5K96C) and water. On day 19, 20 and 21 the rats are dosed subcutaneously with 17 $\alpha$ -ethinyl-17 $\beta$ -estradiol (0.06  $\mu$ g/rat/day), test compound or vehicle (50% DMSO/50% Dulbecco's PBS). To assess estrogen receptor antagonist activity, compounds are coadministered with 17 $\alpha$ -ethinyl-17 $\beta$ -estradiol (0.06  $\mu$ g/rat/day). There are six rats/group and they are euthanized approximately 24 hours after the last injection by CO<sub>2</sub> asphyxiation and pneumothorax. Uteri are removed and weighed after trimming associated fat and expressing any internal fluid. A tissue sample can also be snap frozen for analysis of gene expression (e.g. complement factor 3 mRNA). Results obtained from representative compounds of the invention are shown in Table (3).

Table 3: Evaluation of selected compounds in a rat uterotrophic Test Procedure.

| Compound  | mean uterine weight (mg)<br>$\pm$ SEM |
|---|---------------------------------------|
| Vehicle   | 21.4 $\pm$ 1.59                       |
| 17 $\alpha$ -ethinyl, 17 $\beta$ -estradiol (0.06 $\mu$ g/rat)                        | 85.5 $\pm$ 3.1                        |
| Example 12 (2mg/rat) + 17 $\alpha$ -ethinyl, 17 $\beta$ -estradiol (0.06 $\mu$ g/rat) | 60.2 $\pm$ 4.0                        |
| Example 41 (2mg/rat)  | 30.3 $\pm$ 1.5                        |
| Example 41 (2mg/rat) + 17 $\alpha$ -ethinyl, 17 $\beta$ -estradiol (0.06 $\mu$ g/rat) | 76.6 $\pm$ 3.0                        |

| Compound   | mean uterine weight (mg)<br>± SEM |
|--|-----------------------------------|
| Example 24 (2mg/rat)   | 14.18 ± 1.1                       |
| Example 24 (2mg/rat) + 17 $\alpha$ -ethinyl,17 $\beta$ -estradiol (0.06 $\mu$ g/rat) | 80.7 ± 5.3                        |
| Vehicle  | 30.5 ± 3.2                        |
| 17 $\alpha$ -ethinyl,17 $\beta$ -estradiol (0.06 $\mu$ g/rat)                        | 104.7 ± 5.4                       |
| Example 20 (2mg/rat)   | 39.2 ± 0.7                        |
| Example 20 (2mg/rat) + 17 $\alpha$ -ethinyl,17 $\beta$ -estradiol (0.06 $\mu$ g/rat) | 95.9 ± 5.5                        |
| Example 21 (2mg/rat)   | 38.8 ± 1.7                        |
| Example 21 (2mg/rat) + 17 $\alpha$ -ethinyl,17 $\beta$ -estradiol (0.06 $\mu$ g/rat) | 93.9 ± 5.9                        |

Procedure 2: Sexually immature (18 days of age) 129 SvE mice are obtained from Taconic and provided unrestricted access to a casein-based diet (Purina Mills 5K96C) and water. On day 22, 23, 24 and 25 the mice are dosed subcutaneously with compound or vehicle (corn oil). There are six mice/group and they are euthanized approximately 6 hours after the last injection by CO<sub>2</sub> asphyxiation and pneumothorax. Uteri are removed and weighed after trimming associated fat and expressing any internal fluid. The following results (Table (4)) were obtained for representative compounds from the invention.

Table 4: Evaluation of selected compounds in a mouse uterotrophic Test Procedure.

| Compound                        | mean uterine weight (mg)<br>± SEM |
|---------------------------------|-----------------------------------|
| vehicle                         | 10.2 ± 2.1                        |
| 17 $\beta$ -estradiol (50mg/kg) | 41.7 ± 3.6                        |
| Example 21 (20mg/kg)            | 12.1 ± 1.7                        |
| Vehicle                         | 11.7 ± 0.5                        |
| 17 $\beta$ -estradiol (50mg/kg) | 41.9 ± 2.9                        |
| Example 24 (50mg/kg)            | 10.7 ± 0.9                        |
| Vehicle                         | 9.6 ± 0.4                         |
| 17 $\beta$ -estradiol (50mg/kg) | 40.0 ± 2.0                        |
| Example 34 (50mg/kg)            | 10.3 ± 0.7                        |
| Vehicle                         | 9.4 ± 0.4                         |

| Compound                | mean uterine weight (mg)<br>± SEM |
|-------------------------|-----------------------------------|
| 17β-estradiol (50mg/kg) | 35.6 ± 4.4                        |
| Example 25 (50mg/kg)    | 9.7 ± 1.0                         |
| Vehicle                 | 13.7 ± 2.0                        |
| 17β-estradiol (50mg/kg) | 40.5 ± 5.84                       |
| Example 12 (50mg/kg)    | 13.7 ± 0.82                       |
| Example 20 (50mg/kg)    | 13.1 ± 0.86                       |
| Vehicle                 | 9.6 ± 0.36                        |
| 17β-estradiol (50mg/kg) | 40.0 ± 2.0                        |
| Example 34 (50mg/kg)    | 10.3 ± 0.69                       |
| Vehicle                 | 9.8 ± 1.2                         |
| 17β-estradiol (50mg/kg) | 42.9 ± 4.8                        |
| Example 26 (50mg/kg)    | 9.0 ± 0.3                         |
| Example 42 (50mg/kg)    | 9.5 ± 0.6                         |
| Example 64 (50mg/kg)    | 9.8 ± 0.7                         |

#### Evaluation of osteoporosis and lipid modulation (cardioprotection)

Female Sprague-Dawley rats, ovariectomized or sham operated, are obtained 1 day after surgery from Taconic Farms (weight range 240 - 275 g). They are housed 3 or 4 rats/cage in a room on a 12/12 (light/dark) schedule and provided with food (Purina 5K96C rat chow) and water ad libitum. Treatment for all studies begin 1 day after arrival and rats are dosed 7 days per week as indicated for 6 weeks. A group of age matched sham operated rats not receiving any treatment serve as an intact, estrogen replete control group for each study.

All test compounds are prepared in a vehicle of 50% DMSO (JT Baker, Phillipsburg, NJ) / 1x Dulbecco's phosphate saline (GibcoBRL, Grand Island, NY) at defined concentrations so that the treatment volume is 0.1 mL/100 g body weight. 17β-estradiol is dissolved in corn oil (20 µg/mL) and delivered subcutaneously, 0.1 mL/rat. All dosages are adjusted at three week intervals according to group mean body weight measurements, and given subcutaneously.

Five weeks after the initiation of treatment and one week prior to the termination of the study, each rat is evaluated for bone mineral density (BMD). The total and trabecular density of the proximal tibia are evaluated in anesthetized rats using an XCT-960M (pQCT; Stratec Medizintechnik, Pforzheim, Germany). The

measurements are performed as follows: Fifteen minutes prior to scanning, each rat is anesthetized with an intraperitoneal injection of 45 mg/kg ketamine, 8.5 mg/kg xylazine, and 1.5 mg/kg acepromazine.

5 The right hind limb is passed through a polycarbonate tube with a diameter of 25 mm and taped to an acrylic frame with the ankle joint at a 90° angle and the knee joint at 180°. The polycarbonate tube is affixed to a sliding platform that maintains it perpendicular to the aperture of the pQCT. The platform is adjusted so that the distal end of the femur and the proximal end of the tibia is in the scanning field. A two dimensional scout view is run for a length of 10 mm and a line resolution of 0.2 mm. 10 After the scout view is displayed on the monitor, the proximal end of the tibia is located. The pQCT scan is initiated 3.4 mm distal from this point. The pQCT scan is 1 mm thick, has a voxel (three dimensional pixel) size of 0.140 mm, and consists of 145 projections through the slice.

After the pQCT scan is completed, the image is displayed on the monitor. A 15 region of interest including the tibia but excluding the fibula is outlined. The soft tissue is mathematically removed using an iterative algorithm. The density of the remaining bone (total density) is reported in  $\text{mg}/\text{cm}^3$ . The outer 55% of the bone is mathematically peeled away in a concentric spiral. The density of the remaining bone (Trabecular density) is reported in  $\text{mg}/\text{cm}^3$ .

20 One week after BMD evaluation the rats are euthanized by  $\text{CO}_2$  asphyxiation and pneumothorax, and blood is collected for cholesterol determination. The uteri are also removed and the weighed after trimming associated fat and expressing any luminal fluid. Total cholesterol is determined using a Boehringer-Mannheim Hitachi 911 clinical analyzer using the Cholesterol/HP kit. Statistics were compared using 25 one-way analysis of variance with Dunnet's test.

The following results were obtained with representative compounds of the invention (Table (5)).

Table 5. Evaluation of bone mineral density in the ovariectomized rat after administration of selected compounds of the invention

| Compound                                       | Total Bone Mineral Density (mean mg/cm <sup>3</sup> ± SEM) | Trabecular Bone Mineral Density (mean mg/cm <sup>3</sup> ± SEM) |
|--|--|---|
| Vehicle  | 543.49 ± 14.24   | 353.96 ± 13.46  |
| 17β-estradiol (2μg/rat)                        | 639.49 ± 14.47   | 453.28 ± 24.93  |
| Example 24 (10mg/kg)                           | 517.56 ± 9.67  | 321.16 ± 9.04   |
| Example 21 (10mg/kg)                           | 501.40 ± 11.97   | 312.34 ± 19.73  |
| Example 20 (10mg/kg)                           | 525.51 ± 7.93  | 287.56 ± 17.56  |
| Example 20 (10mg/kg) + 17β-estradiol (2μg/rat) | 682.41 ± 24.01   | 491.43 ± 36.43  |
| Sham operated (no manipulation)                | 685.28 ± 15.68   | 510.96 ± 16.99  |

#### Evaluation of antioxidant activity

Porcine aortas are obtained from an abattoir, washed, transported in chilled PBS, and aortic endothelial cells are harvested. To harvest the cells, the intercostal vessels of the aorta are tied off and one end of the aorta clamped. Fresh, sterile filtered, 0.2% collagenase (Sigma Type I) is placed in the vessel and the other end of the vessel then clamped to form a closed system. The aorta is incubated at 37 °C for 15-20 minutes, after which the collagenase solution is collected and centrifuged for 5 minutes at 2000 x g. Each pellet is suspended in 7 mL of endothelial cell culture medium consisting of phenol red free DMEM/Ham's F12 media supplemented with charcoal stripped FBS (5%), NuSerum (5%), L-glutamine (4mM), penicillin-streptomycin (1000U/ml, 100μg/ml) and gentamycin (75μg/ml), seeded in 100mm petri dish and incubated at 37 °C in 5%CO<sub>2</sub>. After 20 minutes, the cells are rinsed with PBS and fresh medium added, this was repeated again at 24 hours. The cells are confluent after approximately 1 week. The endothelial cells are routinely fed twice a week and, when confluent, trypsinized and seeded at a 1:7 ratio. Cell mediated oxidation of 12.5 μg/mL LDL is allowed to proceed in the presence of the compound to be evaluated (5 μM) for 4 hours at 37 °C. Results are expressed as the percent inhibition of the oxidative process as measured by the TBARS (thiobarbituric acid reactive substances) method for analysis of free aldehydes [Yagi, Biochemical Medicine 15: 212-6 (1976)].

Progesterone receptor mRNA regulation standard pharmacological test procedure

This test procedure can be used to evaluate the estrogenic or antiestrogenic activity of compounds from this invention [Shughrue, et al., Endocrinology 138: 5476-5484 (1997)]. Data for representative compounds of the invention are shown in Table 5 (6).

Table 6. Effect of representative compounds of the invention on regulation of progesterone mRNA in the preoptic area of the rat brain

| Compound<br>(10mg/kg) | Progesterone receptor mRNA<br>(arbitrary units; mean $\pm$ stdev) |
|-----------------------|---|
| Vehicle               | 22.0 $\pm$ 10.1   |
| Example 21            | 110.5 $\pm$ 19.3  |
| Example 20            | 238.6 $\pm$ 36.3  |
| Example 12            | 256.2 $\pm$ 42.3  |
| Vehicle               | 189.2 $\pm$ 27.2  |
| Example 34            | 511.5 $\pm$ 23.7  |
| Example 25            | 447.0 $\pm$ 60.7  |
| Example 26            | 467.8 $\pm$ 66.7  |
| Example 64            | 431.3 $\pm$ 65.6  |

Rat Hot Flush Test Procedure

The effect of test compounds on hot flushes can be evaluated in a standard pharmacological test procedure which measures the ability of a test compound to blunt the increase in tail skin temperature which occurs as morphine-addicted rats are acutely withdrawn from the drug using naloxone [Merchenthaler, et al., Maturitas 30: 307-16 (1998)]. It can also be used to detect estrogen receptor antagonist activity by co-dosing test compound with the reference estrogen. The following data were obtained from representative compounds of the invention (Table (7))

Table 7: Effect of selected compounds of the invention in a rat model of hot flush

| Compound  | Temperature change 15<br>minutes after naloxone<br>injection (mean $\pm$ SEM) |
|---|---|
| Vehicle   | 4.63 $\pm$ 0.79   |
| 17 $\alpha$ -ethinyl, 17 $\beta$ -estradiol<br>(0.3mg/kg) | 2.12 $\pm$ 1.14   |
| Example 20 (15mg/kg)                                      | 5.28 $\pm$ 0.71   |
| Example 41 (15mg/kg)                                      | 5.25 $\pm$ 0.72   |



Evaluation of vasomotor function in isolated rat aortic rings

Sprague-Dawley rats (240-260 grams) are divided into 4 groups:

- 5    1. Normal non-ovariectomized (intact)
2. Ovariectomized (ovex) vehicle treated
3. Ovariectomized 17 $\beta$ -estradiol treated (1 mg/kg/day)
4. Ovariectomized animals treated with test compound (various doses)

10            Animals are ovariectomized approximately 3 weeks prior to treatment. Each animal receives either 17- $\beta$  estradiol sulfate (1 mg/kg/day) or test compound suspended in distilled, deionized water with 1% tween-80 by gastric gavage. Vehicle treated animals received an appropriate volume of the vehicle used in the drug treated groups.

15            Animals are euthanized by CO<sub>2</sub> inhalation and exsanguination. Thoracic aortae are rapidly removed and placed in 37 °C physiological solution with the following composition (mM): NaCl (54.7), KCl (5.0), NaHCO<sub>3</sub> (25.0), MgCl<sub>2</sub> 2H<sub>2</sub>O (2.5), D-glucose (11.8) and CaCl<sub>2</sub> (0.2) gassed with CO<sub>2</sub>-O<sub>2</sub>, 95%/5% for a final pH of 7.4. The adventitia is removed from the outer surface and the vessel is cut into 2-3  
20 mm wide rings. Rings are suspended in a 10 mL tissue bath with one end attached to the bottom of the bath and the other to a force transducer. A resting tension of 1 gram is placed on the rings. Rings are equilibrated for 1 hour, signals are acquired and analyzed.

              After equilibration, the rings are exposed to increasing concentrations of  
25 phenylephrine (10<sup>-8</sup> to 10<sup>-4</sup> M) and the tension recorded. Baths are then rinsed 3 times with fresh buffer. After washout, 200 mM L-NAME is added to the tissue bath and equilibrated for 30 minutes. The phenylephrine concentration response curve is then repeated.

30    Evaluation of cardioprotective activity

              Apolipoprotein E-deficient C57/B1J (apo E KO) mice are obtained from Taconic Farms. All animal procedures are performed under strict compliance to IACUC guidelines. Ovariectomized female apo E KO mice, 4-7 weeks of age, are



housed in shoe-box cages and were allowed free access to food and water. The animals are randomized by weight into groups (n=12-15 mice per group). The animals are dosed with test compounds or estrogen (17 $\beta$ -estradiol sulfate at 1 mg/kg/day) in the diet using a Precise-dosing Protocol, where the amount of diet consumed is measured weekly, and the dose adjusted accordingly, based on animal weight. The diet used is a Western-style diet (57U5) that is prepared by Purina and contains 0.50% cholesterol, 20% lard and 25 IU/KG Vitamin E. The animals are dosed/fed using this paradigm for a period of 12 weeks. Control animals are fed the Western-style diet and receive no compound. At the end of the study period, the animals are euthanized and plasma samples obtained. The hearts are perfused in situ, first with saline and then with neutral buffered 10% formalin solution.

For the determination of plasma lipids and lipoproteins, total cholesterol and triglycerides are determined using enzymatic methods with commercially available kits from Boehringer Mannheim and Wako Biochemicals, respectively and analyzed using the Boehringer Mannheim Hitachi 911 Analyzer. Separation and quantification of plasma lipoproteins were performed using FPLC size fractionation. Briefly, 50-100 mL of serum is filtered and injected into Superose 12 and Superose 6 columns connected in series and eluted at a constant flow rate with 1 mM sodium EDTA and 0.15 M NaCl. Areas of each curve representing VLDL, LDL and HDL are integrated using Waters Millennium™ software, and each lipoprotein fraction is quantified by multiplying the Total Cholesterol value by the relative percent area of each respective chromatogram peak.

For the quantification of aortic atherosclerosis, the aortas are carefully isolated and placed in formalin fixative for 48-72 hours before handling. Atherosclerotic lesions are identified using Oil Red O staining. The vessels are briefly destained, and then imaged using a Nikon SMU800 microscope fitted with a Sony 3CCD video camera system in concert with IMAQ Configuration Utility (National Instrument) as the image capturing software. The lesions are quantified en face along the aortic arch using a custom threshold utility software package (Coleman Technologies). Automated lesion assessment is performed on the vessels using the threshold function of the program, specifically on the region contained within the aortic arch from the proximal edge of the brachio-cephalic trunk to the distal edge of the left subclavian artery. Aortic atherosclerosis data are expressed as percent lesion involvement strictly within this defined luminal area.

Evaluation of cognition enhancement

Ovariectomized rats (n=50) are habituated to an 8-arm radial arm maze for 10-min periods on each of 5 consecutive days. Animals are water-deprived prior to habituation and testing. A 100  $\mu$ L aliquot of water placed at the ends of each arm serves as reinforcement. Acquisition of a win-shift task in the radial arm maze is accomplished by allowing the animal to have access to one baited arm. After drinking, the animal exits the arm and re-enters the central compartment, where it now has access to the previously visited arm or to a novel arm. A correct response is recorded when the animal chooses to enter a novel arm. Each animal is given 5 trials per day for 3 days. After the last acquisition trial, the animals are assigned to one of the following 4 groups:

1. Negative controls: injected with 10% DMSO/ sesame oil vehicle once daily for 6 days (1 mL/kg, SC)
2. Positive controls: injected with 17 $\beta$ -estradiol benzoate for 2 days and tested 4 days after the second injection (17 $\beta$ -estradiol benzoate at 10  $\mu$ g/0.1 mL per rat)
3. Estradiol: 17 $\beta$ -estradiol will be injected daily for 6 days (20  $\mu$ g/kg, SC)
4. Test compound: injected daily for 6 days (doses vary).

All injections will begin after testing on the last day of acquisition. The last injection for groups 1, 3, and 4 will take place 2 hours before testing for working memory.

The test for working memory is a delayed non-matching-to-sample task (DNMS) utilizing delays of 15, 30, or 60 seconds. This task is a variation of the acquisition task in which the rat is placed in the central arena and allowed to enter one arm as before. A second arm is opened once the rat traverses halfway down the first arm, and again the rat is required to choose this arm. When it has traveled halfway down this second arm, both doors are closed and the delay is instituted. Once the delay has expired, both of the original two doors, and a third novel door, are opened simultaneously. A correct response is recorded when the animal travels halfway down the third, novel arm. An incorrect response is recorded when the animal travels halfway down either the first or second arms. Each animal will receive 5 trials at each of the three delay intervals for a total of 15 trials per subject.

Evaluation of effect on pleurisy

The ability to reduce the symptoms of experimentally-induced pleurisy in rats can be evaluated according to the procedure of Cuzzocrea [Endocrinology 141: 1455-63 (2000)].

5

Evaluation of protection against glutamate-induced cytotoxicity (neuroprotection)

The neuroprotective activity of compounds of this invention can be evaluated in an in vitro standard pharmacological test procedure using glutamate challenge [Zaulyanov, et al., Cellular & Molecular Neurobiology 19: 705-18 (1999); Prokai, et al.,  
10 Journal of Medicinal Chemistry 44: 110-4 (2001)].

Evaluation in the Mammary End Bud Test Procedure

Estrogens are required for full ductal elongation and branching of the mammary ducts, and the subsequent development of lobulo-alveolar end buds under  
15 the influence of progesterone. In this test procedure, the mammotrophic activity of selected compounds of the invention was evaluated according to the following standard pharmacological test procedure. Twenty-eight day old Sprague-Dawley rats (Taconic Farms) were ovariectomized and rested for nine days. Animals were housed under a 12-hour light/dark cycle, fed a casein-based Purina Laboratory Rodent Diet  
20 5K96 (Purina, Richmond, IN) and allowed free access to water. Rats were then dosed subcutaneously for six days with vehicle (50% DMSO (JT Baker, Phillipsburg, NJ) / 50% 1x Dulbecco's Phosphate buffered saline (GibcoBRL), 17 $\beta$ -estradiol (0.1 mg/kg) or test compound (20 mg/kg). For the final three days, rats were also dosed subcutaneously with progesterone (30 mg/kg). On the seventh day, rats were  
25 euthanised and a mammary fat pad excised. This fat pad was analyzed for casein kinase II mRNA as a marker of end bud proliferation. Casein kinase II mRNA was analyzed by real-time RT-PCR. Briefly, RNA was isolated following Trizol (GibcoBRL) according to the manufacture's directions, Samples were treated with DNase I using DNA-free kit (Ambion), and casein kinase II mRNA levels were measured by real-time  
30 RT-PCR using the Taqman Gold procedure (PE Applied Biosystems). A total of 50 ng of RNA was analyzed in triplicate using casein kinase II specific primer pair (5' primer, CACACGGATGGCGCATACT; 3' primer, CTCGGGATGCACCATGAAG) and customized probe (TAMRA-CGGCACTGGTTTCCCTCACATGCT-FAM). Casein kinase II mRNA levels were normalized to 18s ribosomal RNA contained within each

sample reaction using primers and probe supplied by PE Applied Biosystems. The following results were obtained for representative compounds of the invention (Table (8)).

| Table 8: Evaluation of compounds in a rat mammotrophic assay |   |
|--|---|
| Compound   | Casein kinase II mRNA/18S rRNA (mean $\pm$ SEM) |
| Vehicle + Progesterone (30mg/kg)                             | 1.61 $\pm$ 0.36                                 |
| 17 $\beta$ -estradiol (0.1mg/kg) + Progesterone (30mg/kg)    | 39.0 $\pm$ 5.36                                 |
| Example 24 (20mg/kg) + Progesterone (30mg/kg)                | 3.98 $\pm$ 0.79                                 |

5

#### Evaluation in the HLA Rat Standard Pharmacological Test Procedure for inflammatory bowel disease

Representative compounds of the invention were evaluated in the HLA rat standard pharmacological test procedure which emulates inflammatory bowel disease in humans. The following briefly describes the procedure used and results obtained. Male HLA-B27 rats were obtained from Taconic and provided unrestricted access to food (PMI Lab diet 5001) and water. Stool quality was observed daily and graded according to the following scale: Diarrhea = 3; soft stool = 2; normal stool = 1. At the end of the study, serum was collected and stored at  $-70^{\circ}\text{C}$ . A section of colon was prepared for histological analysis and an additional segment was analyzed for myeloperoxidase activity.

In Study A, rats (22-26 weeks old) were dosed subcutaneously once per day for seven days with one of the regimens listed below. There were five rats in each group and the last dose was administered two hours before euthanasia.

- Vehicle (50% DMSO/50% Dulbecco's PBS)
- Example 24 (50 mg/kg)

The results from Study A are shown in Table (9). Rats dosed with vehicle continued to have diarrhea throughout the course of the study. Stool quality was improved in rats treated with Example 24.

| Table 9: Evaluation of stool character of HLA rats treated subcutaneously with compounds for 5 days. Value reported is the group's average score. |         |                      |
|---|---------|----------------------|
| Day   | Vehicle | Example 24 (50mg/kg) |
| 1   | 3       | 2.8                  |
| 2   | 3       | 2                    |
| 3   | 3       | 1.8                  |
| 4   | 3       | 1.6                  |
| 5   | 3       | 1.6                  |
| 6   | 3       | 1.4                  |
| 3= diarrhea; 2= soft stool; 1=normal stool  |         |                      |

In Study B, rats (8-10 weeks old) were dosed orally for twenty-six days as follows:

- Vehicle (2% Tween-80/0.5% methylcellulose)
- Example 25 (10 mg/kg from days 1-14; then increased to 20 mg/kg at d15)
- Example 34 (10 mg/kg)

The following results were obtained (Table (10)) and show that stool character improved in all rats treated with representative compounds of the invention.

Table 10: Evaluation of stool character of HLA rats treated orally with vehicle or representative compounds from the invention. Value reported is the group's average score.

| Day | Vehicle | Example 25 | Example 34 |
|-----|---------|------------|------------|
| 1   | 1       | 1          | 1          |
| 2   | 1       | 1          | 1          |
| 3   | 1       | 1.25       | 1          |
| 4   | 1.25    | 1.25       | 1.25       |
| 5   | 2.5     | 1.75       | 2          |
| 6   | 2.75    | 1.5        | 1.75       |
| 7   | 2.75    | 2          | 1.75       |
| 8   | 2.75    | 2          | 1.5        |
| 9   | 3       | 1.75       | 1.5        |
| 10  | 3       | 1.5        | 1.25       |
| 11  | 2.75    | 2          | 1.5        |
| 12  | 2.75    | 1.75       | 1.5        |
| 13  | 2.75    | 2.25       | 1.25       |
| 14  | 2.75    | 2          | 1.25       |
| 15  | 2.75    | 2          | 1.25       |
| 16  | 3       | 1.5        | 1          |
| 17  | 2.75    | 1.5        | 1          |
| 18  | 2.75    | 1.5        | 1.25       |
| 19  | 2.75    | 1.25       | 1          |
| 20  | ND      | ND         | ND         |

| Day  | Vehicle | Example 25 | Example 34 |
|--|---------|------------|------------|
| 21   | ND      | ND         | ND         |
| 22   | 3       | 1.25       | 1          |
| 23   | 3       | 1.25       | 1          |
| 24   | 3       | 1.25       | 1          |
| 25   | 3       | 1.25       | 1          |
| 26   | 3       | 1.25       | 1          |
| ND: Not determined<br>3= diarrhea; 2= soft stool; 1=normal stool |         |            |            |

In Study C, rats (8-10 weeks old) were dosed orally once per day for forty-six days with one of the formulations listed below. There were 4 rats in each group and the last dose was administered two hours before euthanasia.

- Vehicle (2% Tween-80/0.5% methylcellulose)
- Example 21 (10 mg/kg from days 1-18; then increased to 20 mg/kg at d19)
- Example 24 (10 mg/kg from days 1-24; then increased to 20 mg/kg at d25)

The following results were obtained (Table (11)) and show that stool character improved with administration of all the ER $\beta$  selective compounds:

Table 11: Stool scores from HLA rats treated orally with vehicle or representative compounds from the invention. Value reported is the group's average score.

| Day | Vehicle | Example 24 | Example 21 |
|-----|---------|------------|------------|
| 1   | 2.75    | 2.75       | 2.75       |
| 2   | 3       | 2.75       | 3          |
| 3   | 3       | 2.75       | 2.75       |
| 4   | 3       | 2.5        | 2.75       |
| 5   | 3       | 2          | 2.75       |
| 6   | 3       | 2.5        | 2.5        |
| 7   | 3       | 2.25       | 2.5        |
| 8   | 3       | 2.25       | 2.75       |
| 9   | 3       | 2.25       | 2.5        |
| 10  | 3       | 2.25       | 2.75       |
| 11  | 3       | 2.25       | 2.5        |
| 12  | 3       | 1.75       | 2.5        |
| 13  | 3       | 2.25       | 2.5        |
| 14  | 3       | 2          | 2.5        |
| 15  | 3       | 1.75       | 2.5        |
| 16  | 3       | 1.75       | 2.5        |
| 17  | 3       | 1.75       | 2.5        |
| 18  | 3       | 1.75       | 2.5        |
| 19  | 3       | 1.75       | 2.75       |
| 20  | 3       | 1.75       | 2.5        |
| 21  | 3       | 1.75       | 2.75       |
| 22  | 3       | 1.75       | 2.5        |



| Day  | Vehicle | Example 24 | Example 21 |
|--|---------|------------|------------|
| 23   | 3       | 1.75       | 2.25       |
| 24   | 3       | 2          | 1.75       |
| 25   | 3       | 2          | 2          |
| 26   | 2.75    | 2.25       | 2          |
| 27   | 3       | 1.75       | 2          |
| 28   | 3       | 1.75       | 2          |
| 29   | 3       | 1.5        | 2          |
| 30   | 2.75    | 1.5        | 2.25       |
| 31   | 3       | 1.5        | 2.25       |
| 32   | 3       | 1.5        | 2          |
| 33   | 3       | 1.75       | 1.5        |
| 34   | 3       | 1.75       | 1.75       |
| 35   | 3       | 1.5        | 1.5        |
| 36   | 3       | 1.5        | 1.75       |
| 37   | 3       | 1.25       | 1.5        |
| 38   | 3       | 1.75       | 1.5        |
| 39   | 3       | 1.75       | 2          |
| 40   | 3       | 1.5        | 1.75       |
| 41   | 3       | 1.75       | 2          |
| 42   | 3       | 1.5        | 2          |
| 43   | 3       | 1.5        | 2          |
| 44   | 3       | 1.5        | 2          |
| 45   | 3       | 1.25       | 2          |
| 46   | 3       | 1.25       | 2          |
| 3= diarrhea; 2= soft stool; 1=normal stool |         |            |            |

Histological analysis. Colonic tissue was immersed in 10% neutral buffered formalin. Each specimen of colon was separated into four samples for evaluation. The formalin-fixed tissues were processed in a Tissue Tek vacuum infiltration processor (Miles, Inc; West Haven, Connecticut) for paraffin embedding. The samples were sectioned at 5  $\mu$ m and then stained with hematoxylin and eosin (H&E) for blinded histologic evaluations using a scale modified after Boughton-Smith. After the scores were completed the samples were unblinded, and data were tabulated and analyzed by ANOVA linear modeling with multiple mean comparisons. Sections of colonic tissue were evaluated for several disease indicators and given relative scores. As shown in Table (12) (a composite of two subcutaneous dosing studies, including Study A), Example 24 is effective in reducing several measurements of tissue injury.



| Table 12: Histological scoring of disease severity in the HLA-B27 rat model:<br>Composite of two studies using subcutaneous dosing for 5 days. |                     |                       |                          |                   |                |
|--|---------------------|-----------------------|--------------------------|-------------------|----------------|
| Group  | Ulceration<br>(0-2) | Inflammation<br>(0-3) | Lesion<br>depth<br>(0-2) | Fibrosis<br>(0-2) | Total<br>score |
| Vehicle  | 1.38                | 2.69                  | 1.19                     | 0.88              | 6.13           |
| Example 24 (50mg/kg)   | 0.25*#              | 1.05*#                | 0.2#                     | 0*                | 1.5*#          |
| Example 24 (10mg/kg) <sup>a</sup>  | 0.81*               | 1.63*                 | 0.69*                    | 0.50*             | 3.6*           |
| Example 24 (1mg/kg) <sup>a</sup>   | 1.25                | 1.63*                 | 0.88*                    | 0.75              | 4.4*           |
| <sup>a</sup> data taken from a second study<br>* sig < vehicle or EE + ICI<br># sig < EE   |                     |                       |                          |                   |                |

Intestinal tissue from Study B (see above) was also examined histologically. As shown below (Table (13)), both compounds significantly reduced total disease score.

5

| Table 13: Histological scoring of disease severity in the colon from animals treated orally<br>for 4 weeks with representative compounds from the invention . Means $\pm$ SD |                     |                       |                       |                   |                  |
|--|---------------------|-----------------------|-----------------------|-------------------|------------------|
| Group  | Ulceration<br>(0-2) | Inflammation<br>(0-3) | Lesion depth<br>(0-2) | Fibrosis<br>(0-2) | Total score      |
| Vehicle  | 1.44 $\pm$ 0.66     | 2.88 $\pm$ 0.14       | 1.56 $\pm$ 0.63       | 1.06 $\pm$ 0.32   | 6.94 $\pm$ 1.51  |
| Example 25   | 0.44 $\pm$ 0.24*    | 1.50 $\pm$ 0.35*      | 0.44 $\pm$ 0.24*      | 0.31 $\pm$ .13*   | 2.69 $\pm$ 0.52* |
| Example 34   | 0.75 $\pm$ 0.46*    | 1.81 $\pm$ 0.13*      | 0.63 $\pm$ 0.32*      | 0.31 $\pm$ 0.32*  | 3.50 $\pm$ 1.10* |
| * sig < vehicle  |                     |                       |                       |                   |                  |

Intestinal tissue from Study C (see above) was also examined histologically. As shown below (Table (14)), Example 24 significantly reduced total disease score. The scores of Example 21 on all disease parameters, although not statistically significant, were lower than corresponding scores from vehicle-treated rats.

10

| Table 14: Histological scoring of disease severity in the colon from animals treated orally for 7 weeks with representative compounds from the invention.<br>Means $\pm$ SD |                     |                       |                       |                   |                  |
|---|---------------------|-----------------------|-----------------------|-------------------|------------------|
| Group   | Ulceration<br>(0-2) | Inflammation<br>(0-3) | Lesion depth<br>(0-2) | Fibrosis<br>(0-2) | Total score      |
| Vehicle   | 1.19 $\pm$ 0.69     | 2.38 $\pm$ 0.32       | 1.0 $\pm$ 0.54        | 0.94 $\pm$ 0.75   | 5.50 $\pm$ 2.1   |
| Example 21  | 0.81 $\pm$ 0.47     | 2.06 $\pm$ 0.43       | 0.75 $\pm$ 0.50       | 0.56 $\pm$ 0.32   | 4.19 $\pm$ 1.74  |
| Example 24  | 0*                  | 0.69 $\pm$ 0.24*      | 0*                    | 0*                | 0.69 $\pm$ 0.24* |
| * sig < vehicle   |                     |                       |                       |                   |                  |

Evaluation in two models of arthritis

*Lewis rat assay of adjuvant-induced arthritis.* Sixty, female, 12 weeks old, Lewis rats are housed according to standard facility operating procedures. They receive a standard regimen of food and water ad libitum. Each animal is identified  
5 by a cage card indicating the project group and animal number. Each rat number is marked by indelible ink marker on the tail. At least 10-21 days before study they are anesthetized and ovariectomized by standard aseptic surgical techniques.

Freund's Adjuvant-Complete (Sigma Immuno Chemicals, St. Louis, MO) is used to induce arthritis, each mL containing 1 mg Mycobacterium tuberculosis heat  
10 killed and dried, 0.85 mL mineral oil and 0.15 mL mannide monooleate Lot No. 084H8800.

The following are examples of two test procedures. Inhibition test procedure: Thirty rats are injected intradermally with 0.1 mL of Freund's Adjuvant-Complete at the base of the tail. The animals are randomized to four groups, each group containing six  
15 rats. Each day, the groups receive vehicle (50% DMSO (JT Baker, Phillipsburg, NJ) / 1x Dulbecco's phosphate saline (GibcoBRL, Grand Island, NY)) or test compound (administered subcutaneously). All rats began treatment on Day 1. Data for representative compounds of the invention are shown in Table (15).

Treatment test procedure: Thirty rats are injected intradermally with 0.1 mL of  
20 Freund's Adjuvant-Complete at the base of the tail. The animals are randomized to four groups, each group containing six rats. Each day, the groups receive vehicle (50% DMSO (JT Baker, Phillipsburg, NJ) / 1x Dulbecco's phosphate saline (GibcoBRL, Grand Island, NY)) or test compound (administered subcutaneously). All rats began treatment on Day 8 after adjuvant injection. Data for representative  
25 compounds of the invention are shown in Tables (16), (17) and (18).

Statistical analysis was performed using Abacus Concepts Super ANOVA. (Abacus Concepts, Inc., Berkeley, CA). All of the parameters of interest were subjected to Analysis of Variance with Duncan's new multiple range post hoc testing between groups. Data are expressed throughout as mean  $\pm$  standard deviation (SD),  
30 and differences were deemed significant if  $p < 0.05$ .

The degree of arthritis severity is monitored daily in terms of the following disease indices: Hindpaw erythema, hindpaw swelling, tenderness of the joints, and movements and posture. An integer scale of 0 to 3 is used to quantify the level of erythema (0= normal paw, 1= mild erythema, 2= moderate erythema, 3= severe

erythema) and swelling (0=normal paw, 1=mild swelling, 2= moderate swelling, 3= severe swelling of the hind paw). The maximal score per day is 12.

At the end of the study the rats are euthanized with CO<sub>2</sub>, hindlimbs removed at necropsy and fixed in 10% buffered formalin, and the tarsal joints decalcified and embedded in paraffin. Histologic sections are stained with Hematoxylin and Eosin or Saffranin O - Fast Green stain.

Slides are coded so that the examiner is blinded to the treatment groups. Synovial tissue from tarsal joints is evaluated based on synovial hyperplasia, inflammatory cell infiltration, and pannus formation [Poole and Coombs, International Archives of Allergy & Applied Immunology 54: 97-113 (1977)] as outlined below.

| Category  | Grade |
|---|-------|
| 1. Synovial lining cells  |       |
| a. No change  | 0     |
| b. Cells enlarged, slightly thickened   | 1     |
| c. Cells enlarged, increase in numbers, moderately thickened. No villus present                         | 2     |
| d. Cells enlarged, thickened. Villus present  | 3     |
| 2. Fibroplasia  |       |
| a. No change  | 0     |
| b. Fibroplasia present under lining cells   | 1     |
| c. Small areas of areolar tissue replaced by fibrous tissue   | 2     |
| d. Replacement of areolar tissue by fibrous tissue  | 3     |
| 3. Inflammatory cells   |       |
| a. Occasionally seen, scattered throughout selection  | 0     |
| b. Cells present in small numbers in or just under lining cell layer and/or around blood vessels.       | 1     |
| c. Small focal collection of cells may be present   | 2     |
| d. Large numbers of cells present in capsule and in or under lining cell layers. Large foci often seen. | 3     |
| 4. Pannus   |       |
| a. Not detectable   | 0     |
| b. Detectable   | 1     |

In addition, articular cartilage and bone is evaluated using Mankin's histological grading system [Mankin, et al., Journal of Bone & Joint Surgery - American Volume 53: 523-37 (1971)] as shown below.

| Category                           | Grade |
|------------------------------------|-------|
| 1. Structure                       |       |
| a. Normal                          | 0     |
| b. Surface irregularity            | 1     |
| c. Pannus and surface irregularity | 2     |
| d. Clefts to transitional zone     | 3     |
| e. Clefts to radial zone           | 4     |
| f. Clefts to calcified zone        | 5     |
| g. Complete disorganization        | 6     |
| 2. Cells                           |       |
| a. Normal                          | 0     |
| b. Diffuse hypercellularity        | 1     |
| c. Cloning                         | 2     |
| d. Hypocellularity                 | 3     |
| 3. Safranin-O staining             |       |
| a. Normal                          | 0     |
| b. Slight reduction                | 1     |
| c. Modest reduction                | 2     |
| d. Severe reduction                | 3     |
| e. No dye noted                    | 4     |
| 4. Tidemark integrity              |       |
| a. Intact                          | 0     |
| b. Crossed by blood vessels        | 1     |

| Table 15: Evaluation of joint inflammation of Lewis rats:<br>Inhibition protocol |         |            |
|--|---------|------------|
| Day  | Vehicle | Example 24 |
| 1  | 0.00    | 0.00       |
| 2  | 0.00    | 1.00       |
| 3  | 4.50    | 4.50       |
| 4  | 5.50    | 4.83       |
| 5  | 9.33    | 5.83       |
| 6  | 10.50   | 6.16       |
| 7  | 10.60   | 6.16       |
| 8  | 11.00   | 5.33       |
| 9  | 11.50   | 5.66       |
| 10   | 11.33   | 4.33       |
| 11   | 10.83   | 3.16       |
| 12   | 10.83   | 3.16       |
| 13   | 11.00   | 2.16       |
| 14   | 11.00   | 3.33       |
| 15   | 11.00   | 3.00       |
| 16   | 11.00   | 1.66       |
| 17   | 10.50   | 1.50       |

| Table 16: Evaluation of joint inflammation of Lewis rats:<br>Treatment protocol |         |            |            |            |
|---|---------|------------|------------|------------|
| Day   | Vehicle | Example 24 | Example 27 | Example 32 |
| 1   | 10.83   | 11.33      | 11.33      | 11.33      |
| 2   | 11.00   | 11.15      | 11.15      | 10.83      |
| 3   | 10.83   | 11.33      | 11.33      | 9.33       |
| 4   | 11.33   | 9.50       | 9.83       | 8.00       |
| 5   | 11.50   | 8.00       | 8.83       | 5.83       |
| 6   | 11.50   | 7.00       | 7.83       | 3.33       |
| 7   | 11.50   | 5.83       | 6.16       | 3.00       |
| 8   | 11.50   | 4.83       | 5.00       | 2.50       |
| 9   | 11.00   | 3.50       | 4.33       | 2.50       |
| 10  | 11.00   | 3.83       | 2.66       | 2.50       |
| 11  | 10.66   | 3.83       | 1.83       | 2.50       |
| 12  | 10.66   | 3.83       | 1.83       | 2.50       |
| 13  | 10.50   | 3.16       | 2.66       | 2.50       |
| 14  | 9.83    | 3.16       | 2.66       | 2.50       |
| 15  | 8.10    | 2.83       | 2.00       | 2.00       |
| 16  | 7.35    | 2.83       | 2.00       | 1.33       |
| 17  | 6.50    | 2.00       | 1.50       | 1.00       |

| Table 17: Histological scoring of synovitis in the tarsal joints of Lewis rats<br>(mean $\pm$ SD): Treatment protocol |                          |                   |                          |                  |                              |
|---|--------------------------|-------------------|--------------------------|------------------|------------------------------|
| Group   | Synovial Structure (0-3) | Fibroplasia (0-3) | Inflammatory Cells (0-3) | Pannus (0-1)     | Total Synovitis Score (0-10) |
| Vehicle   | 2.58 $\pm$ 0.38          | 1.75 $\pm$ 0.42   | 2.92 $\pm$ 0.20          | 1.00 $\pm$ 0.89  | 8.25 $\pm$ 1.57              |
| Example 24 50mg/kg  | 1.42 $\pm$ 0.49*         | 0.42 $\pm$ 0.80*  | 1.33 $\pm$ 0.41*         | 0.08 $\pm$ 0.20* | 3.25 $\pm$ 1.54*             |
| * sig < vehicle   |                          |                   |                          |                  |                              |

| Table 18: Histological scoring of cartilage change (Mankin scores) in the tarsal joints of Lewis rats (mean $\pm$ SD): Treatment protocol |                           |                       |  |                          |                           |
|---|---------------------------|-----------------------|--|--------------------------|---------------------------|
| Group   | Cartilage Structure (0-6) | Cartilage Cells (0-3) | Saffranin-O/ Fast Green Staining (0-4) | Tidemark Integrity (0-1) | Total Mankin Score (0-14) |
| Vehicle   | 2.83 $\pm$ 0.26           | 2.58 $\pm$ 0.38       | 2.50 $\pm$ 0.32                        | 0                        | 7.92 $\pm$ 0.74           |
| Example 24 50mg/kg  | 1.58 $\pm$ 0.49*          | 0.83 $\pm$ 0.75*      | 1.25 $\pm$ 0.69*                       | 0                        | 3.67 $\pm$ 1.86*          |
| * sig < vehicle   |                           |                       |  |                          |                           |

*Evaluation in the HLA-B27 Rat model of arthritis.* Representative compounds of the invention were evaluated in the HLA-B27 rat standard pharmacological test procedure which emulates arthritis in humans. The following briefly describes the procedure used and results obtained. Male HLA-B27 rats were obtained from Taconic and provided unrestricted access to a food (PMI Lab diet 5001) and water. Joint scores and histology are evaluated as described above for the Lewis rat model of adjuvant-induced arthritis.

Study 1: Rats (8-10 weeks old) were dosed orally once per day for forty-six days with one of the formulations listed below. There were 4 rats in each group and the last dose was administered two hours before euthanasia.

- Vehicle (2% Tween-80/0.5% methylcellulose)
- Example 21 (10 mg/kg from days 1-18; then increased to 20 mg/kg at d19)
- Example 24 (10 mg/kg from days 1-24; then increased to 20 mg/kg at d25)

The following results were obtained for representative compounds of the invention (Tables (19) and (20)).

| Table 19: Evaluation of joint inflammation from Study 1 |         |            |            |
|---|---------|------------|------------|
| Day   | Vehicle | Example 24 | Example 21 |
| 29  | 2.5     | 1.5        | 0.75       |
| 30  | 6       | 0.5        | 1.75       |
| 31  | 5       | 0.5        | 1.25       |
| 32  | 6.75    | 1.25       | 0.75       |
| 33  | 8       | 2          | 1          |
| 34  | 8       | 2.25       | 1.25       |
| 35  | 8       | 2          | 2.25       |
| 36  | 6       | 2.25       | 1          |
| 37  | 7.5     | 2          | 4          |
| 38  | 6.5     | 2.75       | 1.5        |
| 39  | 7.5     | 2.25       | 1.5        |
| 40  | 7.5     | 1.75       | 2.25       |
| 41  | 6.5     | 2          | 2.25       |
| 42  | 6.5     | 2.5        | 1.5        |
| 43  | 6       | 4.75       | 1.25       |
| 44  | 6.75    | 3          | 1          |
| 45  | 5.5     | 2.75       | 2.5        |
| 46  | 6       | 3.25       | 2          |

| Table 20: Evaluation of joint histology from Study 1. |                                 |                              |
|---|---------------------------------|------------------------------|
| Compound  | Synovitis score (mean $\pm$ SD) | Mankin score (mean $\pm$ SD) |
| Vehicle   | 7.75 $\pm$ 2.6                  | 6.75 $\pm$ 1.0               |
| Example 24  | 3.17 $\pm$ 0.3*                 | 3.5 $\pm$ 1.8**              |
| Example 21  | 6.1 $\pm$ 0.75                  | 4.6 $\pm$ 0.9                |
| * sig < vehicle, p < 0.07                             |                                 |                              |
| ** sig < vehicle, p < 0.05                            |                                 |                              |

Study 2: Rats (8-10 weeks old) were dosed orally for twenty-six days with one of the formulations listed below. There were 4 rats in each group and the last dose was administered two hours before euthanasia.

- 5
- Vehicle (2% Tween-80/0.5% methylcellulose)
  - Example 25 (10 mg/kg from days 1-14; then increased to 20 mg/kg at d15)
  - Example 34 (10 mg/kg)

The following results were obtained for representative compounds of the invention (Table (21)).

| Table 21: Evaluation of joint inflammation of HLA rats from Study 2. |         |            |            |
|--|---------|------------|------------|
| Day  | Vehicle | Example 25 | Example 34 |
| 1  | 0       | 0          | 0          |
| 2  | 0       | 0          | 0          |
| 3  | 0       | 0          | 0          |
| 4  | 0       | 0          | 0          |
| 5  | 0       | 0          | 0          |
| 6  | 0       | 0          | 0          |
| 7  | 0       | 0          | 0          |
| 8  | 2.5     | 1          | 0.25       |
| 9  | 3.75    | 2          | 0.75       |
| 10   | 2.75    | 2.25       | 0.5        |
| 11   | 3.5     | 2.25       | 0.5        |
| 12   | 1.25    | 2          | 0.25       |
| 13   | 1.25    | 2          | 0.5        |
| 14   | 1.25    | 2          | 0          |
| 15   | 5.25    | 3.75       | 0.5        |
| 16   | 4.5     | 3          | 0.5        |
| 17   | 3.5     | 2.75       | 0.25       |
| 18   | 3.75    | 2          | 0.75       |
| 19   | 5.5     | 1.5        | 1          |
| 22   | 3.25    | 1.25       | 1          |
| 23   | 6.5     | 2.5        | 1.75       |
| 24   | 6.5     | 2          | 1.75       |
| 25   | 6.25    | 2          | 2          |
| 26   | 7       | 1.75       | 3          |



### Evaluation in in vivo models of carcinogenesis

The ability of compounds of this invention to treat and inhibit various malignancies or hyperproliferic disorders can be evaluated in standard pharmacological test procedures that are readily available in the literature, and include the following two procedures.

*Breast cancer.* Athymic nu/nu (nude) mice are obtained ovariectomized from Charles River Laboratories (Wilmington, MA). One day prior to tumor cell injection, animals are implanted with time-release pellets containing 0.36-1.7 mg 17 $\beta$ -estradiol (60 or 90 day release, Innovative Research of America, Sarasota, FL) or a placebo. The pellet is introduced subcutaneously into the intrascapular region using a 10-gauge precision trochar. Subsequently, mice are injected subcutaneously into the breast tissue with either 1x10<sup>7</sup> MCF-7 cells or 1x10<sup>7</sup> BG-1 cells. The cells are mixed with an equal volume of matrigel, a basement membrane matrix preparation to enhance tumor establishment. Test compounds can be evaluated either by dosing one day after tumor cell implantation (inhibition regimen) or after tumors have reached a certain size (treatment regimen). Compounds are administered either intraperitoneally or orally in a vehicle of 1% tween-80 in saline each day. Tumor size is evaluated every three or seven days.

*Colon cancer.* The ability to treat or inhibit colon cancer can be evaluated in the test procedure of Smirnoff [Oncology Research 11: 255-64 (1999)].

### Evaluation of neuroprotection in two in vivo test procedures

*Transient global ischemia in the Mongolian gerbil.* The effect of test compounds on preventing or treating brain injury in response to oxygen deprivation/reperfusion can be measured using the following test procedure.

Female Mongolian gerbils (60-80 g; Charles River Laboratories, Kingston, NY) were housed in the Wyeth-Ayerst animal care facility (AAALAC certified) with a 12-hour light, 12-hour dark photoperiod and free access to tap water and a low-estrogen casein diet (Purina; Richmond, IN). After acclimation (3-5 days), gerbils were anesthetized with isoflurane (2-3% mixture with O<sub>2</sub>), ovariectomized (Day 0). Beginning the following morning (Day 1), gerbils were treated subcutaneously each day with either vehicle (10% ETOH/corn oil), 17 $\beta$ -estradiol (1 mg/kg, sc) or an experimental compound. On Day 6, gerbils (n=4-5/group) were anesthetized with

isoflurane, the common carotid arteries visualized via a mid-line neck incision and both arteries simultaneously occluded for 5 minutes with non-traumatic micro aneurysm clips. After occlusion, the clips were removed to allow cerebral reperfusion and the neck incision closed with wound clips. All animals were fasted overnight prior to the global ischemia surgery, a step that facilitates consistent ischemic injury. On Day 12, gerbils were exposed to a lethal dose of CO<sub>2</sub>, and the brains frozen on dry ice and stored at -80°C. The animal protocols used for these studies were reviewed and approved by the Radnor/Collegeville Animal Care and Use Committee (RACUC/CACUC) at Wyeth-Ayerst Research.

The degree of neuronal protection was evaluated by in situ hybridization analysis of neurogranin mRNA. Briefly, 20 µm coronal cryostat sections were collected on gelatin-coated slides, dried and stored at -80°C. At the time of processing, the desiccated slide boxes were warmed to room temperature, the slides postfixed in 4% paraformaldehyde, treated with acetic anhydride and then delipidated and dehydrated with chloroform and ethanol. Processed section-mounted slides were then hybridized with 200 µl (6x10<sup>6</sup> DPM/ slide) of an antisense or sense (control) riboprobe for Neurogranin (<sup>35</sup>S-UTP-labeled NG-241; bases 99-340) in a 50% formamide hybridization mix and incubated overnight at 55°C in a humidified slide chamber without coverslipping. The following morning, the slides were collected in racks, immersed in 2xSSC (0.3 M NaCl, 0.03 M sodium citrate; pH 7.0) / 10 mM DTT, treated with RNase A (20 µg/ml) and washed (2 x 30 min) at 67°C in 0.1x SSC to remove nonspecific label. After dehydration, the slides were exposed to BioMax (BMR-1; Kodak) X-ray film overnight.

The level of neurogranin hybridization signal was used to quantitatively assess the degree of neuronal loss in the CA1 region after injury and to evaluate the efficacy of 17β-estradiol and experimental compounds. Neurogranin mRNA was selected for these studies because it is highly expressed in the hippocampal neurons including CA1, but absent in glia and other cell types present in this brain region. Therefore, measurement of the amount of neurogranin mRNA present represents surviving neurons. Relative optical density measurements of neurogranin hybridization signal were obtained from film autoradiograms with a computer based image analysis system (C-Imaging Inc., Pittsburgh, PA). The results from 6 sections (40 µm apart) per animal were averaged and statistically evaluated. Numerical values are reported

as the mean  $\pm$  SEM. One-way analysis of variance was used to test for differences in the level of neurogranin mRNA and all statements of non-difference in the results section imply that  $p > 0.05$ .

The following results were obtained with representative compounds of the invention (Table (22)).

| Table 22: Effect of representative compounds from the invention on preserving neurons in the gerbil hippocampus |   |
|---|---|
| Compound  | Neurogranin mRNA<br>(arbitrary units, mean $\pm$ stdev) |
| Vehicle   | 0.0   |
| Example 24  | 0.0   |
| Example 41  | 43.0 $\pm$ 21.8   |

*Middle cerebral artery occlusion in mice.* Neuroprotection can be evaluated according to the test procedures described by Dubal [see, Dubal, et al., Proceedings of the National Academy of Sciences of the United States of America 98: 1952-1957 (2001), Dubal, et al., Journal of Neuroscience 19: 6385-6393 (1999)].

#### Ovulation inhibition standard pharmacological test procedure

The test procedure is used to determine whether test compounds can inhibit or change the timing of ovulation. It can also be used to determine the number of oocytes ovulated [Lundeen, et al., J Steroid Biochem Mol Biol 78: 137-143 (2001)]. The following data were obtained from representative compounds from the invention (Table (23))

| Table 23: Effect of representative compounds from the invention on inhibiting ovulation. |                                    |
|--|------------------------------------|
| Compound   | Number of oocytes (mean $\pm$ SEM) |
| Vehicle  | 13.00 $\pm$ 0.72                   |
| Example 20 (50 mg/kg)  | 14.13 $\pm$ 0.79                   |
| Example 24 (50 mg/kg)  | 13.86 $\pm$ 0.77                   |

#### Evaluation in an endometriosis standard pharmacologic test procedure

This procedure is slightly modified from a published method [Bruner-Tran. et al., Journal of Clinical Investigation 99: 2851-2857 (1997)]. In brief, normal human

endometrial tissue (cycle day ~12) is treated in vitro overnight with 10nM 17 $\beta$ -estradiol and then implanted into ovariectomized athymic nude mice. For the purposes of these studies, the mice do not receive estrogen/placebo implants as described in the paper. Lesions are allowed to establish for at least 10 days, then oral daily dosing  
5 begins and continues for at least 15 days. It should be noted that all mice have visible lesions at the start of dosing. At necropsy the number of mice with lesions is determined as well as the lesions per mouse.

The compound of Example 24 was evaluated three times in this procedure at a  
10 dose of 10mg/kg. In each test procedure, mice dosed with the compound of Example 24 had fewer lesions at necropsy than those mice dosed with vehicle. For example, in Study 1, each of the four mice in the vehicle group had at least one lesion and there were 10 total lesions in this group. In contrast, only two of six mice treated with Example 24 had any lesions and only one lesion was found per animal. Therefore,  
15 because all mice had lesions at the start of treatment, the compound of Example 24 caused lesion regression in four of six mice

Based on the results obtained in the standard pharmacological test procedures, the compounds of this invention are estrogen receptor modulators useful  
20 in the treatment or inhibition of conditions, disorders, or disease states that are at least partially mediated by an estrogen deficiency or excess, or which may be treated or inhibited through the use of an estrogenic agent. The compounds of this invention are particularly useful in treating a peri-menopausal, menopausal, or postmenopausal patient in which the levels of endogenous estrogens produced are greatly diminished.  
25 Menopause is generally defined as the last natural menstrual period and is characterized by the cessation of ovarian function, leading to the substantial diminution of circulating estrogen in the bloodstream. As used herein, menopause also includes conditions of decreased estrogen production that may be surgically, chemically, or be caused by a disease state which leads to premature diminution or  
30 cessation of ovarian function.

The compounds of this invention are also useful in inhibiting or treating other effects of estrogen deprivation including, hot flushes, vaginal or vulvar atrophy, atrophic vaginitis, vaginal dryness, pruritus, dyspareunia, dysuria, frequent urination, urinary incontinence, urinary tract infections. Other reproductive tract uses include the

treatment or inhibition of dysfunctional uterine bleeding. The compounds are also useful in treating or inhibiting endometriosis.

The compounds of this invention are also active in the brain and are therefore useful for inhibiting or treating Alzheimer's disease, cognitive decline, decreased libido, senile dementia, neurodegenerative disorders, depression, anxiety, insomnia, schizophrenia, and infertility. The compounds of this invention are also useful in treating or inhibiting benign or malignant abnormal tissue growth including, glomerulosclerosis, prostatic hypertrophy, uterine leiomyomas, breast cancer, scleroderma, fibromatosis, endometrial cancer, polycystic ovary syndrome, endometrial polyps, benign breast disease, adenomyosis, ovarian cancer, melanoma, prostate cancer, cancers of the colon, CNS cancers, such as glioma or astioblastoma.

The compounds of this invention are cardioprotective and are antioxidants, and are useful in lowering cholesterol, triglycerides, Lp(a), and LDL levels; inhibiting or treating hypercholesteremia, hyperlipidemia, cardiovascular disease, atherosclerosis, peripheral vascular disease, restenosis, and vasospasm, and inhibiting vascular wall damage from cellular events leading toward immune mediated vascular damage.

The compounds of this invention are also useful in treating disorders associated with inflammation or autoimmune diseases, including inflammatory bowel disease (Crohn's disease, ulcerative colitis, indeterminate colitis), arthritis (rheumatoid arthritis, spondyloarthropathies, osteoarthritis), pleurisy, ischemia/reperfusion injury (e.g. stroke, transplant rejection, myocardial infarction, etc.), asthma, giant cell arteritis, prostatitis, uveitis, psoriasis, multiple sclerosis, systemic lupus erythematosus and sepsis.

The compounds of this invention are also useful in treating or inhibiting ocular disorders including cataracts, uveitis, and macular degeneration and in treating skin conditions such as aging, alopecia, and acne.

The compounds of this invention are also useful in treating or inhibiting metabolic disorders such as type-II diabetes, of lipid metabolism, appetite (e.g. anorexia nervosa and bulimia).

Compounds in this invention are also useful in treating or inhibiting bleeding disorders such as hereditary hemorrhagic telangiectasia, dysfunctional uterine bleeding, and combating hemorrhagic shock.



The compounds of this invention are useful in disease states where amenorrhea is advantageous, such as leukemia, endometrial ablations, chronic renal or hepatic disease or coagulation diseases or disorders.

5 The compounds of this invention can be used as a contraceptive agent, particularly when combined with a progestin.

10 When administered for the treatment or inhibition of a particular disease state or disorder, it is understood that the effective dosage may vary depending upon the particular compound utilized, the mode of administration, the condition, and severity thereof, of the condition being treated, as well as the various physical factors related to the individual being treated. Effective administration of the compounds of this invention may be given at an oral dose of from about 0.1 mg/day to about 1,000 mg/day. Preferably, administration will be from about 10 mg/day to about 600 mg/day, more preferably from about 50 mg/day to about 600 mg/day, in a single dose  
15 or in two or more divided doses. The projected daily dosages are expected to vary with route of administration.

Such doses may be administered in any manner useful in directing the active compounds herein to the recipient's bloodstream, including orally, via implants,  
20 parentally (including intravenous, intraperitoneal, intraarticularly and subcutaneous injections), rectally, intranasally, topically, ocularly (via eye drops), vaginally, and transdermally.

Oral formulations containing the active compounds of this invention may  
25 comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and  
30 microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc,

sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry  
5 starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate,  
10 and triethanolamine. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). The oral formulation may also consist of administering the active ingredient in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed.

In some cases it may be desirable to administer the compounds directly to the  
15 airways in the form of an aerosol.

The compounds of this invention may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in  
20 glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to inhibit the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of  
25 sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol  
30 (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such



administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Transdermal administration may be accomplished through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

The preparation of representative examples of this invention is described below.

#### Example 1

#### 25 2-(5-Hydroxy-1,3-benzoxazol-2-yl) benzene-1,4-diol

##### Step a) *N*-(2,5-Dimethoxyphenyl)-2,5-dimethoxybenzamide.

A mixture of 2,5-dimethoxybenzoic acid (5.0 g, 27.5 mmol) and thionyl chloride (15 mL) was refluxed for 1 h. The volatiles were removed under vacuum. The residue was dissolved in THF (20 mL) and added into a cold (0°C) solution of 2,5-dimethoxyaniline (4.6 g, 30.2 mmol), triethylamine (5 mL, 35.9 mmol) and THF (40 mL). The mixture was stirred for 30 min, poured into water, acidified with HCl (2N) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc 2/1) gave a white solid (8.1 g, 93% yield, m.p. 121-123 °C); MS *m/e* 318 (M+H)<sup>+</sup>.

Analysis for:  $C_{17}H_{19}NO_5$

Calc'd: C, 64.34; H, 6.03; N, 4.41

Found: C, 64.29; H, 5.95; N, 4.44

**5 Step b) 2-(5-Hydroxy-1,3-benzoxazol-2-yl) benzene-1,4-diol.**

A mixture of *N*-(2,5-dimethoxyphenyl)-2,5-dimethoxybenzamide (1.0 g, 3.1 mmol) and pyridine hydrochloride (2.0 g, 17.3 mmol) was stirred at 200 °C for 1 h. The mixture was cooled to room temperature and HCl (10 mL, 2 N) was added. The mixture was then extracted with EtOAc and the organic extracts were dried over  $MgSO_4$ .

10 Evaporation and purification by flash chromatography (hexanes / EtOAc 2/1) gave a white solid (0.8 g, 76% yield, m.p. 309-311°C); MS *m/e* 242 (M-H)<sup>+</sup>.

Analysis for:  $C_{13}H_9NO_4$

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.98; H, 3.71; N, 5.62

15

**Example 2**

**3-(5-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 2,3-dimethoxybenzoic acid. The product

20 was obtained as a tan solid, m.p. 239-241°C; MS *m/e* 244 (M+H)<sup>+</sup>

Analysis for:  $C_{13}H_9NO_4$

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.86; H, 3.90; N, 5.74

**25 Example 3**

**2-(3-Fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 3-fluoro-4-methoxybenzoic acid and was obtained as a white solid, m.p. 262-268°C; MS *m/e* 244 (M-H)<sup>+</sup>.

30 Analysis for:  $C_{13}H_8FNO_3$

Calc'd: C, 63.68; H, 3.29; N, 5.71

Found: C, 64.01; H, 3.25; N, 5.63

**Example 4****2-(3-Chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 3-chloro-4-methoxybenzoic acid and was  
5 obtained as a white solid, m.p. 254-256 °C; MS *m/e* 260 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>8</sub>ClNO<sub>3</sub>

Calc'd: C, 59.67; H, 3.08; N, 5.35

Found: C, 59.59; H, 3.02; N, 5.25

**10 Example 5****2-(2-Chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 2-chloro-4-methoxybenzoic acid and was  
obtained as a white solid, m.p. 253-255°C; MS *m/e* 262 (M+H)<sup>+</sup>.

15 Analysis for: C<sub>13</sub>H<sub>8</sub>ClNO<sub>3</sub>

Calc'd: C, 59.67; H, 3.08; N, 5.35

Found: C, 59.79; H, 2.87; N, 5.36

**Example 6****20 2-(3-Fluoro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 3-fluoro-4-methoxybenzoic acid and was  
obtained as a white solid, m.p. 269-271°C; MS *m/e* 244 (M-H)<sup>+</sup>.

Analysis for: C<sub>17</sub>H<sub>17</sub>NO<sub>3</sub>

25 Calc'd: C, 63.68; H, 3.29; N, 5.71

Found: C, 63.53; H, 3.71; N, 5.38

**Example 7****2-(3-*tert*-Butyl-4-hydroxyphenyl)-1,3-benzoxazol-6-ol**

30 The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 3-*tert*-butyl-4-methoxybenzoic acid and was obtained as a white solid, m.p. 220-222°C; MS *m/e* 284 (M+H)<sup>+</sup>.

Analysis for: C<sub>17</sub>H<sub>17</sub>NO<sub>3</sub>

Calc'd: C, 72.07; H, 6.05; N, 4.94

35 Found: C, 72.03; H, 6.43; N, 4.72

**Example 8****2-(6-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,4-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 2,5-dimethoxybenzoic acid and was obtained as a tan solid, m.p. 278-280 °C; MS *m/e* 244 (M+H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>9</sub>NO<sub>4</sub>

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 64.09; H, 3.14; N, 5.65

**Example 9****3-(6-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 2,3-dimethoxybenzoic acid and was obtained as a tan solid, m.p. 256-258 °C; MS *m/e* 244 (M+H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>9</sub>NO<sub>4</sub>

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.91; H, 3.98; N, 5.72

**Example 10****4-(6-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 3,4-dimethoxybenzoic acid and was obtained as a white solid, m.p. 282-284°C; MS *m/e* 242 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>9</sub>NO<sub>4</sub>

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.57; H, 3.68; N, 5.63

**Example 11****2-(3-Chloro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 3-chloro-4-methoxybenzoic acid and was obtained as an off-white solid, m.p. 254-256°C; MS *m/e* 262 (M+H)<sup>+</sup>.

Analysis for:  $C_{13}H_9NO_4$

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.57; H, 3.68; N, 5.63

**5 Example 12**

**2-(4-Hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 4-methoxybenzoyl chloride and was obtained as a light yellow solid, m.p. 264-267 °C; MS *m/e* 228 (M+H)<sup>+</sup>.

10 Analysis for:  $C_{13}H_9NO_3$

Calc'd: C, 68.72; H, 3.99; N, 6.16

Found: C, 67.87; H, 4.05; N, 6.23

**Example 13**

**15 4-(5-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,5-dimethoxyaniline, and 2,4-dimethoxybenzoic acid and was obtained as a white solid, m.p. greater than 300 °C; MS *m/e* 242 (M-H)<sup>+</sup>.

Analysis for:  $C_{13}H_9NO_4$

20 Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 63.92; H, 3.74; N, 5.56

**Example 14**

**2-(4-Hydroxyphenyl)-1,3-benzoxazol-6-ol**

25 The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 4-methoxybenzoyl chloride and was obtained as a white solid, m.p. greater than 300 °C; MS *m/e* 226 (M-H)<sup>+</sup>.

Analysis for:  $C_{13}H_9NO_3$

Calc'd: C, 68.72; H, 3.99; N, 6.16

30 Found: C, 68.09; H, 4.01; N, 6.05

**Example 15****4-(6-Hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol**

The title compound was prepared in substantially the same manner as described in Example 1, from 2,4-dimethoxyaniline, and 2,4-dimethoxybenzoic acid and was  
5 obtained as a white solid, m.p. 293-296 °C; MS *m/e* 242 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>9</sub>NO<sub>4</sub>

Calc'd: C, 64.20; H, 3.73; N, 5.76

Found: C, 64.43; H, 3.77; N, 5.74

**10 Example 16****6-Chloro-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) N-(4-Chloro-2,5-dimethoxyphenyl)-3-fluoro-4-methoxybenzamide.**

The title compound was prepared in substantially the same manner as described in Example 1, step a, from 4-chloro-2,5-dimethoxyaniline, and 3-fluoro-4-  
15 methoxybenzoic acid and was obtained as a white solid, m.p. 197-199 °C; MS *m/e* 340 (M+H)<sup>+</sup>.

Analysis for: C<sub>16</sub>H<sub>15</sub>ClFNO<sub>4</sub>

Calc'd: C, 56.56; H, 4.45; N, 4.12

Found: C, 56.33; H, 4.35; N, 4.05

**20****Step b) N-(4-Chloro-2,5-dihydroxyphenyl)-3-fluoro-4-hydroxybenzamide.**

Boron trifluoride dimethyl sulfide complex (70 mL) was added into a mixture of N-(4-chloro-2,5-dimethoxyphenyl)-3-fluoro-4-methoxybenzamide (1.75 g, 5.15 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (35 mL). After stirring for 20 h, the solvent and the excess reagent were  
25 evaporated under a nitrogen stream in the hood. The residue was taken in a mixture of ice and HCl (1N) and extracted with EtOAc. The organic layer was washed with HCl (1N) and dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/hexanes/EtOAc 5/3/2, and AcOH 10 mL per 1 liter of the eluting solvent) gave a white solid (1.4 g, 91% yield, m.p. 254-256 °C); MS *m/e* 296  
30 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>9</sub>ClFNO<sub>4</sub>

Calc'd: C, 52.46; H, 3.05; N, 4.71

Found: C, 51.98; H, 2.98; N, 4.56

**Step c) 6-Chloro-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 1, step b, from *N*-(4-chloro-2,5-dihydroxyphenyl)-3-fluoro-4-hydroxybenzamide and pyridine hydrochloride and was obtained as a white solid, m.p. 258-260 °C;

5 MS *m/e* 278 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>17</sub>ClFNO<sub>3</sub>

Calc'd: C, 55.83; H, 2.52; N, 5.01

Found: C, 55.35; H, 2.59; N, 4.91

10 **Example 17**

**6-Bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 16, from 4-bromo-2,5-dimethoxyaniline, and 3-fluoro-4-methoxybenzoic acid and was obtained as a white solid, m.p. 224-226 °C; MS *m/e* 322 (M-H)<sup>+</sup>.

15 Analysis for: C<sub>13</sub>H<sub>17</sub>BrFNO<sub>3</sub>

Calc'd: C, 48.18; H, 2.18; N, 4.32

Found: C, 48.69; H, 2.36; N, 4.59

**Example 18**

20 **6-Chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 16, from 4-chloro-2,5-dimethoxyaniline, and 4-methoxybenzoyl chloride and was obtained as an off-white solid, m.p. 260-262 °C; MS *m/e* 260 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>8</sub>ClNO<sub>3</sub>

25 Calc'd: C, 59.67; H, 3.08; N, 5.35

Found: C, 59.09; H, 3.06; N, 5.11

**Example 19****5-Chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-6-ol**

30 The title compound was prepared in substantially the same manner as described in Example 16, from 5-chloro-2,4-dimethoxyaniline, and 4-methoxybenzoyl chloride and was obtained as an off-white solid, m.p. 254-256 °C; MS *m/e* 262 (M+H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>8</sub>ClNO<sub>3</sub>

Calc'd: C, 59.67; H, 3.08; N, 5.35

35 Found: C, 59.40; H, 2.97; N, 5.22



**Example 20****7-Bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) 2-Bromo-4-methoxy-6-nitrophenol.**

- 5 Bromine (16.0 g, 100 mmol) in acetic acid (20 mL) was added into a mixture of 4-methoxy-2-nitrophenol (16.9 g, 100 mmol, sodium acetate (16.4 g, 200 mmol) and acetic acid (100 mL). The mixture was stirred for 30 min at room temperature, and then at 70°C for 2 h and poured into water (1.5 l) containing concentrated sulfuric acid (10 mL). The precipitated solid filtered and crystallized from (chloroform / hexane) to  
10 give a brownish solid, m.p. 116-118 °C; MS *m/e* 246 (M-H)<sup>+</sup>.

Analysis for: C<sub>7</sub>H<sub>6</sub>BrNO<sub>4</sub>

Calc'd: C, 33.90; H, 2.44; N, 5.65

Found: C, 34.64; H, 2.16; N, 5.43

15 **Step b) 2-Amino-6-bromo-4-methoxyphenol.**

- Raney/Ni (2.5 g) was added into a solution of 2-bromo-4-methoxy-6-nitrophenol (8.8 g, 35.5 mmol) in EtOAc (100 mL). The mixture was shaken in a Parr apparatus under hydrogen at 25psi for 2.5 h. The reaction mixture was filtered through celite and concentrated under vacuum to give a gray solid (7.4 g, 96% yield; 95-97°C); MS *m/e*  
20 218 (M+H)<sup>+</sup>.

Analysis for: C<sub>7</sub>H<sub>8</sub>BrNO<sub>2</sub>

Calc'd: C, 38.56; H, 3.70; N, 6.42

Found: C, 38.32; H, 3.77; N, 6.24

25 **Step c) 2-Bromo-4-methoxy-6-[(4-methoxybenzoyl)amino]phenyl-4-methoxybenzoate**

- Anhydrous pyridine (37.0 mL, 468.5 mmol) was added dropwise into a cold (0°C) mixture (mechanically stirred) of 2-amino-6-bromo-4-methoxyphenol (20.0 g, 91.7 mmol), 4-methoxybenzoyl chloride (38.9 g, 229.0 mmol), and CH<sub>2</sub>Cl<sub>2</sub> (250 mL).  
30 During the pyridine addition a precipitate was formed. The mixture was stirred for 30 min and then ethyl ether (250 mL) was added. The precipitated solids were filtered off and washed with ethyl ether. The solids were taken in water and stirred for 20 min. The solids were then filtered off and dried to give an off-white solid (42.5 g, 95% yield, m.p. 73-75°C); MS *m/e* 484 (M-H)<sup>+</sup>.

Analysis for:  $C_{23}H_{20}BrNO_6$

Calc'd: C, 56.80; H, 4.15; N, 2.88

Found: C, 56.50; H, 3.78; N, 2.83

5 **Step d) 7-Bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole.**

Route a)

10 A suspension of 2-bromo-4-methoxy-6-[(4-methoxybenzoyl)amino]phenyl 4-methoxybenzoate (42.0 g, 86.4 mmol), *p*-toluenesulfonic acid monohydrate (32.8 g, 172.8 mmol) and anhydrous *p*-xylene (800 mL) was refluxed for 1 h with continuous water removal (Dean-Stark Trap). The initial suspension turned into a brown solution at refluxing temperature. The mixture was cooled to room temperature and washed with NaOH (2N). The organic layer was dried over  $MgSO_4$ . Evaporation and crystallization from acetone/ethyl ether gave an off-white solid (23.5 g, 82% yield, m.p. 139-141°C); MS *m/e* 334 ( $M+H$ )<sup>+</sup>.

15 Analysis for:  $C_{15}H_{12}BrNO_3$

Calc'd: C, 53.91; H, 3.62; N, 4.19

Found: C, 53.83; H, 3.37; N, 4.01

Route b)

20 A mixture of 2-amino-6-bromo-methoxyphenol (100 mg, 0.46 mmol), 4-methoxybenzoic acid (77 mg, 0.5 mmol), and boric acid (31 mg, 0.5 mmol) in *p*-xylene (9 mL) was refluxed for 24 h using a Dean-Stark water separator. The mixture was cooled to room temperature, and concentrated under vacuum. The residual product was purified by flash chromatography (30% EtOAc/petroleum ether) to give a light pink solid (99 mg, 65% yield, m.p. 136-138 °C); MS *m/e* 334 ( $M+H$ )<sup>+</sup>.

25 Analysis for:  $C_{15}H_{12}BrNO_3$

Calc'd: C, 53.91; H, 3.62; N, 4.19

Found: C, 53.78; H, 3.55; N, 4.01.

30 **Step e) 7-Bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol.**

Route a)

Boron tribromide (1M, 89.9 mL, 89.8 mmol) was added dropwise into a cold (-70 °C) suspension of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (10.0 g, 29.94 mmol) and  $CH_2Cl_2$  (50 mL). The mixture was allowed to warm up to room

temperature. During the warming up period the suspension turned into a dark solution. The mixture was stirred at room temperature for 2 days and then poured slowly into cold (0 ° C) ethyl ether (1000 mL). Methyl alcohol (200 mL) was added slowly into the new mixture over a 20 min period. The mixture was then poured into  
5 water (1.5 l). The organic layer was washed three times with water, and dried over MgSO<sub>4</sub>. Evaporation and crystallization from acetone/ethyl ether/hexanes gave an off-white solid (8.4 g, 92% yield, m.p. 298-299 °C); MS *m/e* 306 (M+H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>8</sub>BrNO<sub>3</sub>

Calc'd: C, 51.01; H, 2.63; N, 4.58

10 Found: C, 50.96; H, 2.30; N, 4.42

Route b)

Boron tribromide (0.25 mL, 2.7 mmol) was added dropwise into a cold (-78°C) mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (130 mg, 0.39 mmol),  
15 and dichloromethane (1.5 mL). The reaction mixture was allowed to come gradually to room temperature and stirred for 1 h. The mixture was poured into ice and extracted with EtOAc. The organic extracts were washed with brine and dried over MgSO<sub>4</sub>. Evaporation and flash chromatography (30%-40% EtOAc/petroleum ether) gave (102 mg, 86% yield) of the product as a light pink solid, m.p. 295-298°C; MS *m/e*  
20 304 (M-H)<sup>+</sup>;

Analysis for: C<sub>13</sub>H<sub>8</sub>BrNO<sub>3</sub>

Calc'd: C, 51.01; H, 2.63; N, 4.58

Found: C, 51.06; H, 2.77; N, 4.36.

## 25 Example 21

### 7-Bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol

**Step a) 2-Bromo-6-[(3-fluoro-4-methoxybenzoyl)amino]-4-methoxyphenyl 3-fluoro-4-methoxybenzoate.**

A mixture of 3-fluoro-4-methoxybenzoic acid (39.0 g, 229 mmol), thionyl chloride (100  
30 mL), and N,N-dimethylformamide (0.5 mL) was refluxed for 1 h. The volatiles were removed under vacuum. The solids were taken in benzene (twice) and the volatiles were removed under vacuum. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and added into a cold (0 °C) mixture (mechanically stirred) of 2-amino-6-bromo-4-methoxyphenol (20.0 g, 91.7 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (150 mL). Anhydrous pyridine (37.0

mL, 468.5 mmol) was added dropwise into the new mixture. During the pyridine addition a precipitate was formed. The mixture was stirred for 30 min and then ethyl ether (250 mL) was added. The precipitated solids were filtered off and washed with ethyl ether. The solids were taken in water and stirred for 20 min. The solids were then filtered off and dried to give an off-white solid (46.5 g, 97% yield, m.p. 184-186°C); MS *m/e* 520 (M-H)<sup>+</sup>.

Analysis for: C<sub>23</sub>H<sub>18</sub>BrF<sub>2</sub>NO<sub>6</sub>

Calc'd: C, 52.89; H, 3.47; N, 2.68

Found: C, 52.79; H, 3.23; N, 2.63

**Step b) 7-Bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole.**

A suspension of 2-bromo-6-[(3-fluoro-4-methoxybenzoyl)amino]-4-methoxyphenyl 3-fluoro-4-methoxybenzoate (46.0 g, 88.1 mmol), *p*-toluenesulfonic acid monohydrate (33.5 g, 177.2 mmol) and anhydrous *p*-xylene (1 l) was refluxed for 3 h with continuous water removal (Dean Stark Trap). The initial suspension turned into a brown solution at refluxing temperature. The solids were filtered off and washed with ethyl ether. The solids were suspended in ethyl ether (200 mL), stirred for 10 min, filtered off and dried to give a tan solid (25.1 g, m.p. 175-177°C). The ethyl ether layer was concentrated to 20 mL and 2.5 g of additional product was obtained (90% overall yield). MS *m/e* 352 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>11</sub>BrFNO<sub>3</sub>

Calc'd: C, 51.16; H, 3.15; N, 3.98

Found: C, 51.10; H, 2.92; N, 3.89

**Step c) 7-Bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 20, Step e, and was obtained as a white solid, m.p. 265-267 °C; MS *m/e* 332 (M-H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>7</sub>BrFNO<sub>3</sub>

Calc'd: C, 48.18; H, 2.18; N, 4.32

Found: C, 48.19; H, 2.29; N, 4.19

**Example 22****7-Bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) 2-Fluoro-4-methoxybenzoic acid.**

- Into a warm (55°C) mixture of Ag<sub>2</sub>O (13.5 g, 58.4 mmol), NaOH (19.5 g, 487 mmol) and water (200 mL) was added 2-fluoro-4-methoxybenzaldehyde (15 g, 97.4 mmol). The mixture was stirred for 1 h, filtered off and the precipitated solids were washed with hot water (10 mL). The filtrate was added slowly into cold (0°C) HCl (5N) with vigorous stirring. The precipitated solid was filtered, washed with water and dried to give a white solid (13.6 g, 82% yield, m.p. 194-196 °C); MS *m/e* 169 (M-H)<sup>+</sup>.
- Analysis for: C<sub>8</sub>H<sub>7</sub>FO<sub>3</sub>  
Calc'd: C, 56.48; H, 4.15  
Found: C, 56.12; H, 4.12

**Step b) 7-Bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

- The title compound was prepared in substantially the same manner as described in Example 21, from 2-fluoro-4-methoxybenzoic acid, and was obtained as a white solid, m.p. 248-250 °C; MS *m/e* 324 (M+H)<sup>+</sup>.
- Analysis for: C<sub>13</sub>H<sub>7</sub>BrFNO<sub>3</sub>  
Calc'd: C, 48.18; H, 2.18; N, 4.32  
Found: C, 47.89; H, 1.95; N, 4.18

**Example 23****7-Bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) Methyl 2,3-difluoro-4-methoxybenzoate**

- Iodomethane (10.7 mL, 172.5 mmol) was added into a mixture of 2,3-difluoro-4-hydroxybenzoic acid (10.0 g, 57.5 mmol), lithium carbonate (12.7 g, 172.5 mmol) and N,N-dimethylformamide (100 mL). The mixture was stirred at 40 °C for 12 h, and then poured into water and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc 5/1) gave a white solid (10.2 g, 88% yield, m.p. 66-68 °C); MS *m/e* 203 (M+H)<sup>+</sup>.
- Analysis for: C<sub>9</sub>H<sub>8</sub>F<sub>2</sub>O<sub>3</sub>  
Calc'd: C, 53.47; H, 3.99  
Found: C, 53.15; H, 3.83

**Step b) 2,3-Difluoro-4-methoxybenzoic acid.**

Sodium hydroxide (2N, 50 mL) was added into a mixture of methyl 2,3-difluoro-4-methoxybenzoate (10.0 g, 49.5 mmol), THF (100 mL) and MeOH (100 mL). The mixture was stirred at room temperature for 6 h, and acidified with HCl (2N). The precipitated solid was filtered off, washed with water and dried to give a white solid (8.9 g, 96% yield, m.p. 194-196 °C); MS *m/e* 187 (M-H)<sup>+</sup>.

Analysis for: C<sub>8</sub>H<sub>6</sub>F<sub>2</sub>O<sub>3</sub>

Calc'd: C, 51.08; H, 3.21

Found: C, 50.83; H, 2.92

**Step c) 7-Bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 21, from 2,3-difluoro-4-methoxybenzoic acid, and was obtained as a white solid, m.p. 258-260 °C; MS *m/e* 342 (M+H)<sup>+</sup>.

Analysis for: C<sub>13</sub>H<sub>6</sub>BrF<sub>2</sub>NO<sub>3</sub>

Calc'd: C, 45.64; H, 1.77; N, 4.09

Found: C, 45.33; H, 1.62; N, 4.02

**Example 24****2-(3-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol****Route a)****Step a) 7-Bromo-5-[[*tert*-butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]-3-fluorophenyl)-1,3-benzoxazole.**

*tert*-Butyl(chloro)dimethylsilane (23.2 g, 154 mmol) was added portionwise into a mixture of 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol (16.6 g, 51.4 mmol), imidazole (17.5 g, 257 mmol), *N,N*-dimethylpyridin-4-amine (1.0 g, 8.1 mmol) and DMF (300 mL). The mixture was stirred for 3 h, poured into water and extracted with ethyl ether. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc 50/1) gave a white solid (27.5 g, 97% yield, m.p. 98-99 °C); MS *m/e* 552 (M+H)<sup>+</sup>.

Analysis for: C<sub>25</sub>H<sub>35</sub>BrFNO<sub>3</sub>Si<sub>2</sub>

Calc'd: C, 54.34; H, 6.38; N, 2.53

Found: C, 54.06; H, 6.52; N, 2.24



**Step b) 5-[[*tert*-Butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]-3-fluorophenyl)-7-vinyl-1,3-benzoxazole.**

Dichlorobis(tri-*o*-tolylphosphine)palladium (II) (0.63 g, 0.79 mmol) was added into a mixture of 7-bromo-5-[[*tert*-butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]-3-fluorophenyl)-1,3-benzoxazole (14.7 g, 26.6 mmol), tributyl(vinyl)tin (10.5 g, 33.25 mmol) and *p*-xylene (85 mL). The reaction mixture was stirred at 90°C for 24 h, cooled to room temperature, diluted with ethyl ether (100 mL) and treated with activated carbon. The mixture was filtered through MgSO<sub>4</sub> and concentrated. Purification by flash chromatography (hexanes / EtOAc 50/1) gave a white solid (11.8 g, 89% yield, m.p. 93-95 °C); MS *m/e* 500 (M+H)<sup>+</sup>.

Analysis for: C<sub>27</sub>H<sub>38</sub>FNO<sub>3</sub>Si<sub>2</sub>

Calc'd: C, 64.89; H, 7.66; N, 2.80

Found: C, 64.59; H, 7.70; N, 2.73

**Step c) 2-(3-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.**

Hydrofluoric acid (48 wt.% in water, 1 mL) was added into a solution of 5-[[*tert*-butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]-3-fluorophenyl)-7-vinyl-1,3-benzoxazole (1.5 g, 3.0 mmol), THF (6 mL) and acetonitrile (3 mL). The reaction mixture was stirred at 65 °C for 8 h, and then poured into water. The precipitated solid was filtered off and dried. Crystallization of the product from acetone/ethyl ether gave a white solid (0.72 g, 81% yield, m.p. 249-251°C); MS *m/e* 272 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>10</sub>FNO<sub>3</sub>

Calc'd: C, 66.42; H, 3.72; N, 5.16

Found: C, 66.31; H, 3.85; N, 4.96

**Route b)**

**2-(3-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.**

Dichlorobis(tri-*o*-tolylphosphine)palladium (II) (0.87 g, 1.1 mmol) was added into a mixture of 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol (7.16 g, 22.1 mmol), tributyl(vinyl)tin (10.5 g, 33.25 mmol) and ethylene glycol diethyl ether (65 mL). The reaction mixture was stirred at 115 °C for 48 h, cooled to room temperature and treated with activated carbon. The mixture was filtered through MgSO<sub>4</sub> and concentrated. Purification by flash chromatography, on acidic silica gel (hexanes /



EtOAc / CH<sub>2</sub>Cl<sub>2</sub> 1/1/1), gave a white solid (4.35 g, 72% yield, m.p. 250-252 °C); MS *m/e* 272 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>10</sub>FNO<sub>3</sub>

Calc'd: C, 66.42; H, 3.72; N, 5.16

5 Found: C, 66.03; H, 3.68; N, 5.09

#### Route c)

##### Step a) 4-[5-(Acetyloxy)-7-bromo-1,3-benzoxazol-2-yl]-2-fluorophenyl acetate.

10 Acetic anhydride (1.0 mL, 9.95 mmol) was added into a cold (0°C) solution of 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol (1.24 g, 3.8 mmol), *N,N*-dimethylpyridin-4-amine (1.1 g, 9.18 mmol) and 1,4-dioxane (13 mL). The reaction mixture was allowed to warm up to room temperature and stirred for 20 h. Water (50 mL) was added to the reaction mixture extracted with EtOAc and dried over MgSO<sub>4</sub>. Evaporation and crystallization from EtOAc/hexane gave an off-white solid (0.87 g, 15 56% yield); MS *m/e* 408 (M+H)<sup>+</sup>.

Analysis for: C<sub>17</sub>H<sub>11</sub>BrFNO<sub>5</sub>

Calc'd: C, 50.02; H, 2.72; N, 3.43

Found: C, 49.58; H, 2.59; N, 3.37

##### 20 Step b) 2-[4-(Acetyloxy)-3-fluorophenyl]-7-vinyl-1,3-benzoxazol-5-yl acetate.

Dichlorobis(tri-*o*-tolylphosphine)palladium (II) (46 mg, 0.06 mmol) was added into a mixture 4-[5-(acetyloxy)-7-bromo-1,3-benzoxazol-2-yl]-2-fluorophenyl acetate (0.8 g, 1.98 mmol), tributyl(vinyl)tin (0.9 g, 2.8 mmol) and *p*-xylene (9 mL). The reaction mixture was stirred at 130 °C for 5 h, cooled to room temperature, diluted with ethyl 25 ether (10 mL) and treated with activated carbon. The mixture was filtered through MgSO<sub>4</sub> and concentrated. Purification by flash chromatography (hexanes / EtOAc 5/1) gave a white solid (0.4 g, 56% yield, m.p. 154-156 °C); MS *m/e* 356 (M+H)<sup>+</sup>.

Analysis for: C<sub>19</sub>H<sub>14</sub>FNO<sub>5</sub>

Calc'd: C, 64.23; H, 3.97; N, 3.94

30 Found: C, 63.94; H, 3.78; N, 3.76

##### Step c) 2-(3-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.

Potassium carbonate (55 mg) was added into a solution of 2-[4-(acetyloxy)-3-fluorophenyl]-7-vinyl-1,3-benzoxazol-5-yl acetate (0.14 g, 0.39 mmol) and 1,4-dioxane

(3 mL). The mixture was stirred at 90 °C for 1 h, poured into water, acidified with HCl (2N) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and crystallization from EtOAc/hexanes, gave a white solid (0.06 g, 46% yield, m.p. 250-252 °C); MS *m/e* 272 (M+H)<sup>+</sup>.

- 5 Analysis for: C<sub>15</sub>H<sub>10</sub>FNO<sub>3</sub>  
Calc'd: C, 66.42; H, 3.72; N, 5.16  
Found: C, 66.32; H, 3.47; N, 5.18

#### Example 25

##### 10 2-(2-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.

The title compound was prepared in substantially the same manner as described in Example 24, Route a), from 7-bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, and was obtained as a white solid, m.p. 274-275 °C; MS *m/e* 272 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>10</sub>FNO<sub>3</sub>

- 15 Calc'd: C, 66.42; H, 3.72; N, 5.16  
Found: C, 66.18; H, 3.47; N, 4.97

#### Example 26

##### 2-(2,3-Difluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.

- 20 The title compound was prepared in substantially the same manner as described in Example 24, Route b), from 7-bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, and was obtained as an off-white solid, m.p. 276-278°C; MS *m/e* 290 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>9</sub>F<sub>2</sub>NO<sub>3</sub>

- 25 Calc'd: C, 62.29; H, 3.14; N, 4.84  
Found: C, 61.90; H, 3.05; N, 4.52

#### Example 27

##### 2-(4-Hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.

- 30 The title compound was prepared in substantially the same manner as described in Example 24, Route b), from 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol and was obtained as a white solid, m.p. 249-250 °C; MS *m/e* 254 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>11</sub>NO<sub>3</sub>

- 35 Calc'd: C, 70.99; H, 4.39; N, 5.52  
Found: C, 70.75; H, 4.34; N, 5.46

**Examples 28 and 29**

**4-Bromo-2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (Ex. 28) and 4,6-Dibromo-2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (Ex. 29).**

- 5 *N*-Bromosuccinimide (0.49 g, 2.77 mmol) was added into a mixture of 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (0.75 g, 2.77 mmol) and acetonitrile (30 mL). The reaction mixture was stirred at room temperature for 16 h, poured into water and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc / CH<sub>2</sub>Cl<sub>2</sub> 2/1/1) gave (a) as  
10 a white solid (0.45 g, m.p. 226-228 °C); MS *m/e* 349 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>9</sub>BrNO<sub>3</sub>

Calc'd: C, 51.45; H, 2.59; N, 4.00

Found: C, 51.08; H, 2.40; N, 3.90

and (b) as a white solid (0.18 g, m.p. 272-274 °C); MS *m/e* 428 (M+H)<sup>+</sup>.

- 15 Analysis for: C<sub>15</sub>H<sub>8</sub>Br<sub>2</sub>NO<sub>3</sub>

Calc'd: C, 41.99; H, 1.88; N, 3.26

Found: C, 42.25; H, 1.90; N, 3.14

**Example 30**

- 20 **7-(1,2-Dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

**Step a) 5-Methoxy-2-(4-methoxyphenyl)-7-vinyl-1,3-benzoxazole.**

The title compound was prepared in substantially the same manner as described in Example 24, Route c), step b) from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole and was obtained as a white solid, MS *m/e* 282 (M+H)<sup>+</sup>.

- 25 Analysis for: C<sub>17</sub>H<sub>15</sub>NO<sub>3</sub>

Calc'd: C, 72.58; H, 5.37; N, 4.98

Found: C, 72.33; H, 5.26; N, 4.72

**Step b) 7-(1,2-Dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol.**

- 30 Boron tribromide (0.85 mL, 8.95 mmol) was added dropwise into a cold (-78°C) mixture of 5-methoxy-2-(4-methoxyphenyl)-7-vinyl-1,3-benzoxazole (0.31 g, 1.12 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (4 mL). The mixture was allowed to warm up to room temperature. After stirring for 18 h at room temperature the mixture was slowly poured into cold (0°C) ethyl ether (20 mL). Methyl alcohol (10 mL) was then slowly added into the  
35 mixture. The new mixture was washed with water (three times) and dried over

MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc 3/1) gave a light yellow solid (0.27 g, 59% yield, m.p. 175-177 °C); MS *m/e* 412 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>11</sub>Br<sub>2</sub>NO<sub>3</sub>

Calc'd: C, 43.62; H, 2.68; N, 3.39

5 Found: C, 43.85; H, 2.44; N, 3.33

#### Example 31

##### 7-(1-Bromovinyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol

10 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.25g, 1.65mmol) was added into a solution of 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (0.4 g, 0.96 mmol) and acetonitrile (4 mL). The reaction mixture was stirred for 24 h, poured into cold (0°C) HCl (1N, 10 mL) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub> / hexanes / isopropyl alcohol 15/5/1) gave a white solid (185 mg, 58% yield, m.p. 228-230°C); MS  
15 *m/e* 332 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>10</sub>BrNO<sub>3</sub>

Calc'd: C, 54.24; H, 3.03; N, 4.22

Found: C, 54.27; H, 2.94; N, 4.20

#### 20 Example 32

##### 7-(1-Bromovinyl)-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol

The title compound was prepared in substantially the same manner as described in Examples 29-30, from 7-bromo-2-(2-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole and was obtained as an off-white solid, m.p. 235-237°C; MS *m/e* 350  
25 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>9</sub>BrFNO<sub>3</sub>

Calc'd: C, 51.45; H, 2.59; N, 4.00

Found: C, 51.63; H, 2.38; N, 3.98

#### 30 Example 33

##### 7-(1-Bromovinyl)-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol

The title compound was prepared in substantially the same manner as described in Examples 29-30, from 7-bromo-2-(2,3-difluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole and was obtained as an off-white solid, m.p. 240-242 °C; MS *m/e* 366  
35 (M-H)<sup>+</sup>.

Analysis for:  $C_{15}H_8BrF_2NO_3$

Calc'd: C, 48.94; H, 2.19; N, 3.80

Found: C, 49.63; H, 2.33; N, 3.61

5 **Example 34**

**7-Allyl-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 24, Route c, step b, from 7-bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole, allyltributyltin and dichlorobis(tri-*o*-tolylphosphine)palladium, followed  
10 by demethylation according to Example 20, step e. The desired product was obtained as a light pink solid, m.p. 169-171 °C; MS *m/e* 284 (M-H)<sup>+</sup>.

Analysis for:  $C_{16}H_{12}FNO_3$

Calc'd: C, 67.37; H, 4.24; N, 4.91

Found: C, 67.37; H, 4.16; N, 4.66

15

**Example 35**

**7-Ethynyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

Tetrakis(triphenylphosphine)palladium(0) (52 mg, 0.045 mmol) was added into a mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (0.3 g, 0.9  
20 mmol), copper(I) iodide (17.1 mg, 0.09 mmol), ethynyl(trimethyl)silane (0.2 g mg, 2 mmol) and triethylamine (12 mL). The mixture was stirred at 110°C for 4 h, poured into aqueous ammonium chloride and extracted with EtOAc/THF (1/1). The organic extracts were dried over  $MgSO_4$ . Evaporation and purification by flash chromatography (hexanes/EtOAc 6/1) gave an off-white solid (0.27 g, 85% yield).  
25 The product was dissolved in  $CH_2Cl_2$  (2 mL), cooled to -78°C and boron tribromide (0.6 mL) was added dropwise. The mixture was allowed to warm up to room temperature. After stirring for 18 h at room temperature the mixture was slowly poured into cold (0 °C) ethyl ether (10 mL). Methyl alcohol (3 mL) was then slowly added into the mixture. The new mixture was washed with water (three times) and  
30 dried over  $MgSO_4$ . Evaporation and purification by flash chromatography (hexanes / EtOAc 3/1) gave a yellow solid (86 mg, 38% yield, m.p. 229-231°C); MS *m/e* 252 (M+H)<sup>+</sup>.

Analysis for:  $C_{15}H_9NO_3$

Calc'd: C, 71.71; H, 3.61; N, 5.58

35 Found: C, 71.39; H, 3.49; N, 5.32

**Example 36****2-(4-Hydroxyphenyl)-7-propyl-1,3-benzoxazol-5-ol**

Tetrakis(triphenylphosphine)palladium(0) (70 mg, 0.06 mmol) was added into a mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (0.4 g, 1.2 mmol), bromo(propyl)zinc (0.5 M in THF, 3.6 mL, 1.8 mmol), and THF (4 mL). The mixture was stirred at room temperature for 48 h, poured into HCl (1N) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes/EtOAc 6/1) gave an off-white solid (0.14 g). The product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL), cooled to -78 °C and boron tribromide (0.35 mL) was added dropwise. The mixture was allowed to warm up to room temperature. After stirring for 18 h at room temperature the mixture was slowly poured into cold (0 °C) ethyl ether (10 mL). Methyl alcohol (3 mL) was then slowly added into the mixture. The new mixture was washed with water (three times) and dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / EtOAc 4/1) gave a white solid (90 mg, 27% yield, m.p. 110-112°C); MS *m/e* 270 (M+H)<sup>+</sup>.

Analysis for: C<sub>16</sub>H<sub>15</sub>NO<sub>3</sub>

Calc'd: C, 71.36; H, 5.61; N, 5.20

Found: C, 71.02; H, 5.58; N, 4.94

**Example 37****7-Butyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 35, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole, and bromo(butyl)zinc. The desired product was obtained as a white solid, m.p. 125-127°C; MS *m/e* 282 (M-H)<sup>+</sup>.

Analysis for: C<sub>17</sub>H<sub>17</sub>NO<sub>3</sub>

Calc'd: C, 72.07; H, 6.05; N, 4.94

Found: C, 72.78; H, 5.87; N, 4.69

**Example 38****7-Cyclopentyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared in substantially the same manner as described in Example 35, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole, and



bromo(cyclopentyl)zinc. The desired product was obtained as a white solid, m.p. 220-222 °C; MS *m/e* 296 (M+H)<sup>+</sup>.

Analysis for: C<sub>18</sub>H<sub>17</sub>NO<sub>3</sub>

Calc'd: C, 73.20; H, 5.80; N, 4.74

5 Found: C, 73.05; H, 5.74; N, 4.59

### Example 39

#### Ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate

10 **Step a) 7-Bromo-5-[[*tert*-butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]phenyl)-1,3-benzoxazole.**

The title compound was prepared in substantially the same manner as described in Example 24, Route a, Step a, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole, and *tert*-butyl(chloro)dimethylsilane. The desired product was obtained as a white solid, m.p. 90-91°C; MS *m/e* 534 (M+H)<sup>+</sup>.

15 Analysis for: C<sub>25</sub>H<sub>36</sub>BrNO<sub>3</sub>Si<sub>2</sub>

Calc'd: C, 56.16; H, 6.79; N, 2.62

Found: C, 55.66; H, 6.86; N, 2.68

#### Step b) Ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate.

20 n-Butyllithium (2.5 M, 0.3 mL, 0.75 mmol) was added dropwise into a cold (0 °C) solution of 7-bromo-5-[[*tert*-butyl(dimethyl)silyl]oxy]-2-(4-[[*tert*-butyl(dimethyl)silyl]oxy]phenyl)-1,3-benzoxazole (0.4 g, 0.75 mmol) and THF (4 mL). The mixture was allowed to warm up to 40°C, and then stirred for 2 h. [(Cyanocarbonyl)oxy]ethane (84 mg) in THF (1 ML) was added into the reaction mixture and the reaction mixture was  
25 allowed to warm up to 0°C and stirred for 1 h. The reaction was quenched with aqueous ammonium chloride, extracted with EtOAc, and dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / CH<sub>2</sub>Cl<sub>2</sub> /isopropyl alcohol 18/2/1) gave a colorless oil (340 mg). The product was dissolved in THF (3.5 mL) and treated with tetrabutylammonium fluoride (1M in THF, 1.4 mL). The mixture  
30 was stirred for 30 min poured into HCl (1N) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (hexanes / CH<sub>2</sub>Cl<sub>2</sub> /isopropyl alcohol 5/2/1) gave a white solid (119 mg, 53% yield, m.p. 305-307 °C); MS *m/e* 300 (M+H)<sup>+</sup>.



Analysis for:  $C_{16}H_{13}NO_5$

Calc'd: C, 64.21; H, 4.38; N, 4.68

Found: C, 64.04; H, 4.43; N, 4.40

5 **Example 40**

**2-(4-Hydroxyphenyl)-7-phenyl-1,3-benzoxazol-5-ol**

**Step a) 5-Methoxy-2-(4-methoxyphenyl)-7-phenyl-1,3-benzoxazole**

7-Bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (200 mg, 0.60 mmol) and tetrakis(triphenylphosphine)palladium(0) (63 mg, 0.03 mmol) were dissolved in  
10 toluene (5 mL) and stirred for 10 min at room temperature under a nitrogen atmosphere. Benzene boronic acid (110 mg, 0.90 mmol) was added, followed by aqueous sodium carbonate (2 M, 1.5 mL) and ethanol (2 mL). The mixture was refluxed for 12 h, diluted with water and extracted with EtOAc. The organic extracts  
15 were dried over  $MgSO_4$ . Evaporation and purification by flash chromatography (20% - 40% EtOAc/petroleum ether) gave the title compound as a light pink solid, mp 92°C; MS  $m/e$  332 ( $M+H$ )<sup>+</sup>.

Analysis for  $C_{21}H_{17}NO_3$

Calc'd: C, 76.12; H, 5.17; N, 4.23

Found: C, 75.86; H, 5.08; N, 4.07

20

**Step b) 2-(4-Hydroxyphenyl)-7-phenyl-1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 20, Step e (Route b), and was obtained as a purple solid, m.p. 255-258°C; MS  $m/e$  302 ( $M-H$ )<sup>+</sup>.

Analysis for  $C_{19}H_{13}NO_3 \times 0.25 H_2O$

25 Calc'd: C, 74.14; H, 4.42; N, 4.55

Found: C, 73.81; H, 4.40; N, 4.35

**Example 41**

**5-Hydroxy-2-(4-Hydroxyphenyl)-1,3-benzoxazol-7-carbonitrile**

30 **Step a) 5-Methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbonitrile.**

A solution of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (200 mg, 0.60 mmol), in anhydrous N,N dimethylformamide (1.5 mL) was stirred and heated to reflux under dry nitrogen with copper(I) cyanide (80 mg, 0.90 mmol) for 4 h. The mixture was cooled and poured into an excess of aqueous ethylenediaminetetraacetic acid.

Isolation of the crude product gave the nitrile (164 mg, 98% yield) as tan needles from (30% EtOAc/petroleum ether); m.p. 180-183 °C; MS *m/e* 281 (M+H)<sup>+</sup>.

Analysis for C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> x 0.2 H<sub>2</sub>O

Calcd: C, 66.84; H, 4.48; N, 9.74

5 Found: C, 66.63; H, 4.33; N, 9.60

#### Step b) 5-Hydroxy-2-(4-Hydroxyphenyl)-1,3-benzoxazol-7-carbonitrile

The title compound was prepared according to the procedure of Example 20, Step e (Route b), and was obtained as a light pink solid, mp 297-303°C; MS *m/e* 253 (M+H)<sup>+</sup>.

10 Analysis for C<sub>14</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub> x 0.5 H<sub>2</sub>O

Calcd: C, 64.37; H, 3.47; N, 10.72

Found: C, 64.44; H, 3.49; N, 9.92

#### Example 42

##### 15 5-Hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-carboxamide

The title compound was isolated as a minor product from the reaction of Example 40, Step b, as a light tan solid, m.p. 325°C; MS *m/e* 271 (M+H)<sup>+</sup>.

Analysis for C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub> x 0.5 H<sub>2</sub>O

Calcd: C, 60.22; H, 3.97; N, 10.03

20 Found: C, 59.71; H, 3.91; N, 9.84

#### Example 43

##### 2-(4-Hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol

25 A mixture of 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (100 mg, 0.33 mmol), and copper(I) bromide (56 mg, 0.39 mmol) in anhydrous N,N dimethylformamide (1.5 mL) was stirred with freshly prepared sodium methoxide (15 wt % in methanol, 1 ml) and heated to 120°C for 4 h. The mixture was cooled and diluted with HCl (1N, 5 ml). Isolation of the crude product with ethyl acetate followed by flash chromatography (40% - 50% EtOAc/petroleum ether) gave the title compound as an off-white solid (50  
30 mg, 60% yield, mp 225 - 228 °C); MS *m/e* 258 (M+H)<sup>+</sup>.

Analysis for C<sub>14</sub>H<sub>11</sub>NO<sub>4</sub> x 0.75 H<sub>2</sub>O

Calcd: C, 62.11; H, 4.65; N, 5.17

Found: C, 62.53; H, 4.73; N, 5.02.

**Example 44****7-Ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) 7-Ethyl-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole.**

n-Butyllithium (2.5 N, 0.43 mL, 1.08 mmol) was added dropwise into a cold (-78°C) mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (300 mg, 0.90 mmol) and THF (2 mL). The mixture was allowed to stir for 0.5 h. Iodoethane (0.14 mL, 1.8 mmol) was added dropwise into the mixture. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with aqueous ammonium chloride, poured into water, and extracted with EtOAc. The organic extracts were washed with brine and dried over  $\text{MgSO}_4$ . Evaporation and flash chromatography (20% EtOAc/petroleum ether) gave (231 mg, 91% yield) of the product as a light brown solid: m.p. 85°C; MS  $m/e$  284 (M+H)<sup>+</sup>.

Analysis for:  $\text{C}_{17}\text{H}_{17}\text{NO}_3 \times 0.2 \text{ H}_2\text{O}$

Calc'd: C, 70.28; H, 6.17; N, 4.94.

Found: C, 70.12; H, 5.74; N, 4.82.

**Step b) 7-Ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 20, Step e (Route b) and was obtained as a light brown solid (98% yield), m.p. 110 -115 °C; MS  $m/e$  256 (M+H)<sup>+</sup>.

**Example 45****7-Ethyl-2-(2-ethyl-4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) 7-Ethyl-5-methoxy-2-(2-ethyl-4-methoxyphenyl)-1,3-benzoxazole**

The title compound was prepared according to the procedure of Example 43, Step a, employing two equivalents of n-butyllithium and the crude product was used directly in the next step.

**Step b) 7-Ethyl-2-(2-ethyl-4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared from 7-ethyl-5-methoxy-2-(2-ethyl-4-methoxyphenyl)-1,3-benzoxazole according to the procedure of Example 20, Step e (Route b), and was obtained as a gray solid (87% yield); MS  $m/e$  284 (M+H)<sup>+</sup>.

**Example 46****5-Hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carbaldehyde****Step a) 5-Methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbaldehyde.**

The title compound was prepared according to the procedure of Example 43, Step a, employing N-methylformanilide as the electrophile to give a light orange solid (94%, m.p. 153 - 155°C); MS *m/e* 284 (M+H)<sup>+</sup>.

Analysis for: C<sub>16</sub>H<sub>13</sub>NO<sub>4</sub>

Calc'd: C, 67.84; H, 4.63; N, 4.94

Found: C, 67.58; H, 4.53; N, 4.75

**Step b) 5-Hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carbaldehyde**

The title compound was prepared from 5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbaldehyde according to the procedure of Example 20, Step e (Route b) and was obtained as a dark yellow solid (99% yield, m.p. 273 - 275°C); MS *m/e* 256 (M+H)<sup>+</sup>.

Analysis for C<sub>14</sub>H<sub>9</sub>NO<sub>4</sub> x 0.25 H<sub>2</sub>O

Calcd.: C, 64.74; H, 3.69; N, 5.39

Found: C, 64.32; H, 3.59; N, 5.18.

**Example 47****7-(Hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol****Step a) 5-Methoxy-7-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-benzoxazole**

Sodium borohydride (66.8 mg, 1.76 mmol) was added into a solution of 5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbaldehyde (250 mg, 0.88 mmol) in anhydrous MeOH (8 mL) at 0°C. The reaction mixture was stirred for 30 min and then evaporated in vacuum. The residue was dissolved in diethyl ether and washed with water and brine, dried over MgSO<sub>4</sub> and filtered. Evaporation and flash chromatography (50% EtOAc/petroleum ether) gave (210 mg, 83%) of the product, which was used directly in the next reaction.

**Step b) 7-(Hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol.**

The title compound was prepared from 5-methoxy-7-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-benzoxazole according to the procedure of Example 20, Step e (Route b), and was obtained as a light brown solid, m.p. 282 °C (dec); MS *m/e* 258 (M+H)<sup>+</sup>.

Analysis for  $C_{14}H_{11}NO_4 \times 0.5 H_2O$

Calcd.: C, 63.16; H, 4.54; N, 5.26

Found: C, 63.33; H, 4.36; N, 5.04

5 **Example 48**

**7-(Bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 20, step e (Route b), from 5-methoxy-7-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-benzoxazole with prolonged stirring in the presence of boron tribromide, and was obtained as a light brown solid, m.p. 250 - 260 °C (dec); MS  $m/e$  321 (M+H)<sup>+</sup>.

Analysis for  $C_{14}H_{10}BrNO_3$

Calcd: C, 52.52; H, 3.15; N, 4.38

Found: C, 52.26; H, 3.17; N, 4.07

15 **Example 49**

**[5-Hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-yl] acetonitrile**

To a solution of 7-(bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (122 mg, 0.40 mmol) in N,N-dimethylformamide (1.5 mL) was added 18-crown-6-ether (202 mg, 0.80 mmol) and potassium cyanide (131 mg, 2 mmol). The reaction mixture was allowed to stir for 2 h and then poured into water and extracted with EtOAc. The organic extracts were washed with brine and dried over  $MgSO_4$ . Evaporation and flash chromatography (50%-60% EtOAc/petroleum ether) gave (80 mg, 75% yield) of the product as a gray solid, m.p. 170-180°C; MS  $m/e$  265 (M-H)<sup>+</sup>. Analysis for  $C_{15}H_{10}N_2O_3 \times 1.5 H_2O$

Calcd: C, 61.43; H, 4.47; N, 9.55

Found: C, 61.41; H, 4.21; N, 9.19

**Example 50**

**7-(1-Hydroxy-1-methylethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol]**

30 **Step a) 2-[5-Methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-yl] propan-2-ol**

The title compound was prepared according to the procedure of Example 43, Step a, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole, employing acetone

as the electrophile, to give a white solid (78% yield, m.p. 149 °C); MS *m/e* 314 (M+H)<sup>+</sup>.

Analysis for: C<sub>18</sub>H<sub>19</sub>NO<sub>4</sub>

Calc'd: C, 68.99; H, 6.11; N, 4.47.

5 Found: C, 68.78; H, 6.13; N, 4.35.

**Step b) 7-(1-Hydroxy-1-methylethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol]**

10 The title compound was prepared from 2-[5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-yl] propan-2-ol according to the procedure of Example 20, Step e (Route b), and was obtained as a dark brown solid (90% yield, m.p. 180-185°C); MS *m/e* 286 (M+H)<sup>+</sup>.

Analysis for C<sub>16</sub>H<sub>15</sub>NO<sub>4</sub> x 0.5 H<sub>2</sub>O

Calc'd.: C, 65.30; H, 5.48; N, 4.76

Found: C, 65.03; H, 5.20; N, 4.72

15

**Example 51**

**2-(4-Hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol**

Pyridine hydrochloride (400 mg) was heated to 190 °C. To the melt was added 2-[5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-yl] propan-2-ol (114mg, 0.36 mmol) and the reaction was stirred for 2 h. The mixture was cooled to room temperature, dissolved in water and extracted with EtOAc. The organic layers were combined and washed with HCl (1N), water then brine and dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (50%-60% EtOAc/petroleum ether) gave (40 mg, 41% yield) of the product as a light red-brown solid, m.p. 225-228 °C; MS *m/e* 268 (M+H)<sup>+</sup>.

25

Analysis for C<sub>16</sub>H<sub>13</sub>NO<sub>3</sub> x 0.5 H<sub>2</sub>O

Calc'd.: C, 69.56; H, 5.11; N, 5.06

Found: C, 69.46; H, 5.22; N, 4.56

30 **Example 52**

**2-(4-Hydroxyphenyl)-7-isopropyl-1,3-benzoxazol-5-ol]**

2-(4-Hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol (64 mg, 0.24 mmol) was dissolved in a mixture of EtOAc (5 mL) and absolute ethanol (5 mL) and placed under an inert atmosphere with argon. To this mixture was added 10% Pd-C (25 mg). The



solution was hydrogenated on a Parr apparatus at 25 psi for 3 h. The solution was filtered through celite and rinsed with ethanol. The filtrate was concentrated and the residue purified by flash chromatography (50% EtOAc/petroleum ether) to give (58 mg, 90% yield) of the product as a tan solid, m.p. 200 °C; MS *m/e* 270 (M+H)<sup>+</sup>.

5

**Example 53****7-Bromo-2-(4-hydroxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-ol**

**Step a) 2-Bromo-4-methoxy-6-[[4-methoxy-3-(trifluoromethyl)benzoyl]amino} phenyl 4-methoxy-3-(trifluoromethyl)benzoate.**

10 The title compound was prepared in substantially the same manner as described in Example 20, Step c, from 2-amino-6-bromo-4-methoxyphenol and 4-methoxy-3-trifluoromethyl benzoyl chloride. The product was obtained as an off-white solid, m.p. 205-208 °C; MS *m/e* 622 (M+H)<sup>+</sup>.

Analysis for: C<sub>25</sub>H<sub>18</sub>BrF<sub>6</sub>NO<sub>6</sub>

15 Calc'd: C, 48.25; H, 2.92; N, 2.25

Found: C, 48.47; H, 2.76; N, 2.16

**Step b) 7-Bromo-5-methoxy-2-(4-methoxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazole.**

20 The title compound was prepared in substantially the same manner as described in Example 20, Step d (Route a), from 2-bromo-4-methoxy-6-[[4-methoxy-3-(trifluoromethyl)benzoyl]amino} phenyl 4-methoxy-3-(trifluoromethyl)benzoate and *p*-toluene-sulfonic acid monohydrate. The product was obtained as an off-white solid, m.p. 183-185 °C; MS *m/e* 402 (M+H)<sup>+</sup>.

25 Analysis for: C<sub>16</sub>H<sub>11</sub>BrF<sub>3</sub>NO<sub>3</sub>

Calc'd: C, 47.79; H, 2.76; N, 3.48

Found: C, 47.60; H, 2.50; N, 3.37

**Step c) 7-Bromo-2-(4-hydroxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-ol**

30 The title compound was prepared according to the procedure of Example 20, Step e (Route b), from 7-bromo-5-methoxy-2-(4-methoxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazole, and was obtained as a light yellow solid (50% yield, m.p. 200-210 °C); MS *m/e* 372 (M-H)<sup>+</sup>.

Analysis for  $C_{14}H_7BrF_3NO_3 \times 0.5 H_2O$

Calcd: C, 43.89; H, 2.10; N, 3.65

Found: C, 43.59; H, 2.04; N, 3.6

5 **Example 54**

**7-(2-Furyl)-2-(4-hydroxyphenyl) -1,3-benzoxazol-5-ol**

**Step a) 7-(2-Furyl)-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole**

7-Bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (300 mg, 0.90 mmol) and dichlorobis(tri-*o*-tolylphosphine)palladium(II) (71 mg, 0.09 mmol) were dissolved in *p*-xylene (3 mL) and stirred for 10 min at room temperature under a nitrogen atmosphere. 2-(Tributylstannyl)furan (449 mg, 1.26 mmol) was added and the mixture was refluxed for 4 hours. The mixture was cooled to room temperature, diluted with a saturated solution of ammonium chloride and extracted with EtOAc. The organic extracts were washed with water, then brine and dried over  $MgSO_4$  and concentrated. Purification by flash chromatography (20% - 30% EtOAc/petroleum ether) gave the title compound as a white solid (99% yield, m.p. 120-121 °C); MS *m/e* 322 ( $M+H$ )<sup>+</sup>.

Analysis for  $C_{19}H_{15}NO_4$

Calcd: C, 71.02; H, 4.71; N, 4.36

20 Found: C, 70.23; H, 4.7; N, 4.19

**Step b) 7-(2-Furyl)-2-(4-hydroxyphenyl) -1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 50 and was obtained as a light pink solid (64% yield, m.p. 283-287 °C); MS *m/e* 294 ( $M+H$ )<sup>+</sup>.

25 Analysis for  $C_{17}H_{11}NO_4$

Calcd: C, 69.62; H, 3.78; N, 4.78

Found: C, 69.11; H, 3.6; N, 4.64

**Example 55**

30 **2-(3-Fluoro-4-hydroxyphenyl)- 7-(2-furyl)-1,3-benzoxazol-5-ol**

**Step a) 2-(3-Fluoro-4-methoxyphenyl)- 7-(2-furyl)-5-methoxy-1,3-benzoxazole.**

The title compound was prepared according to the procedure of Example 53, Step a, from 7-bromo-5-methoxy-2-(4-methoxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazole, and was obtained as amber crystals (73% yield, m.p. 155 °C); MS *m/e* 340 ( $M+H$ )<sup>+</sup>.

Analysis for  $C_{19}H_{14}FNO_4$

Calcd: C, 67.25; H, 4.16; N, 4.13

Found: C, 66.88; H, 3.97; N, 4.04

5 **Step b) 2-(3-Fluoro-4-hydroxyphenyl)-7-(2-furyl)-1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 50, from 2-(3-fluoro-4-methoxyphenyl)-7-(2-furyl)-5-methoxy-1,3-benzoxazole, and was obtained as a gray solid (81% yield, m.p. 245-250 °C); MS  $m/e$  312 (M+H)<sup>+</sup>.

Analysis for  $C_{17}H_{10}FNO_4 \times 0.7 C_3H_6O$

10 Calcd: C, 65.04; H, 4.37; N, 3.79

Found: C, 64.84; H, 4.29; N, 3.70

**Example 56**

**2-(4-Hydroxyphenyl)-7-thien-2-yl-1,3-benzoxazol-5-ol**

15 **Step a) 5-Methoxy-2-(4-methoxyphenyl)-7-thien-2-yl-1,3-benzoxazole**

The title compound was prepared according to the procedure of Example 53, Step a, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole and 2-(tributylstannyl)thiophene. The product was obtained as a white solid (95% yield), m.p. 95-100°C; MS  $m/e$  338 (M+H).

20

**Step b) 2-(4-Hydroxyphenyl)-7-thien-2-yl-1,3-benzoxazol-5-ol**

The title compound was prepared according to the procedure of Example 50, from 5-methoxy-2-(4-methoxyphenyl)-7-thien-2-yl-1,3-benzoxazole and was obtained as a gray solid (80% yield, m.p. 278 - 280°C); MS  $m/e$  310 (M+H)<sup>+</sup>.

25 Analysis for  $C_{17}H_{11}NO_3S \times 0.25 H_2O$

Calcd: C, 65.06; H, 3.69; N, 4.46

Found: C, 64.93; H, 3.84; N, 4.21

**Example 57**

30 **2-(4-Hydroxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazol-5-ol**

**Step a) 5-methoxy-2-(4-methoxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazole.**

The title compound was prepared according to the procedure of Example 53, Step a, from 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole and 2-(tributyl-

stannyl)thiazole. The product was obtained as an off white solid (93% yield, m.p. 132-136 °C); MS  $m/e$  339 (M+H)<sup>+</sup>.

Analysis for C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>S

Calcd: C, 63.89; H, 4.17; N, 8.28

5 Found: C, 63.53; H, 3.94; N, 8.15

**Step b) 2-(4-Hydroxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazol-5-ol.**

The title compound was prepared according to the procedure of Example 50, from 5-methoxy-2-(4-methoxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazole, and was obtained  
10 as a yellow solid (55% yield, m.p. 245-255 °C); MS  $m/e$  311 (M+H)<sup>+</sup>.

Analysis for C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>S x 1.5 H<sub>2</sub>O

Calcd: C, 56.97; H, 3.88; N, 8.30

Found: C, 57.24; H, 3.95; N, 7.50

15 **Example 58**

**2-(3-Fluoro-4-hydroxyphenyl)-5-hydroxy-1,3-benzoxazole-7-carbonitrile**

The title compound was prepared according to the procedure of Example 35, from 7-bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole, and zinc cyanide. The product was obtained as a white solid, m.p. 308-310 °C, MS  $m/e$  269 (M-H)<sup>+</sup>.

20 Analysis for C<sub>14</sub>H<sub>7</sub>FN<sub>2</sub>O<sub>3</sub> x 1.5 H<sub>2</sub>O

Calcd: C, 61.01; H, 2.77; N, 10.16

Found: C, 60.68; H, 2.46; N, 9.77

**Examples 59 and 60**

25 **4-Bromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol (Ex. 59)**

**4,6-Dibromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol (Ex. 60)**

The title compounds were prepared according to the procedure of Example 28, from 2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol, and N-bromosuccinimide. Product (a) was obtained as a white solid, m.p. 246-248 °C, MS  $m/e$  336 (M+H)<sup>+</sup>.

30 Analysis for C<sub>14</sub>H<sub>10</sub>BrNO<sub>4</sub> x .1 H<sub>2</sub>O

Calcd: C, 49.49; H, 3.08; N, 4.12

Found: C, 49.28; H, 2.89; N, 3.87.

Product (b) was obtained as a white solid, m.p. 260-262 °C, MS *m/e* 414 (M+H)<sup>+</sup>.

Analysis for C<sub>14</sub>H<sub>9</sub>Br<sub>2</sub>NO<sub>4</sub>

Calcd: C, 40.52; H, 2.19; N, 3.37

Found: C, 40.21; H, 2.00; N, 3.3

5

### Example 61

7-Bromo-2-(3,5-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol

The title compound was prepared in substantially the same manner as described in Example 21, from 3,5-difluoro-4-methoxybenzoic acid, and 2-amino-6-bromo-4-methoxyphenol, and was obtained as a white solid, m.p. 270-272 °C; MS *m/e* 340 (M-H)<sup>+</sup>.

10

Analysis for: C<sub>13</sub>H<sub>6</sub>BrF<sub>2</sub>NO<sub>3</sub>

Calc'd: C, 45.64; H, 1.77; N, 4.09

Found: C, 45.81; H, 1.73; N, 3.89

15

### Example 62

2-(3,5-Difluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol

The title compound was prepared in substantially the same manner as described in Example 24, Route b, from 7-bromo-2-(3,5-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol, and was obtained as a white solid, m.p. 160-262 °C; MS *m/e* 288 (M-H)<sup>+</sup>.

20

Analysis for: C<sub>15</sub>H<sub>9</sub>F<sub>2</sub>NO<sub>3</sub> x 0.1 H<sub>2</sub>O

Calc'd: C, 61.52; H, 3.23; N, 4.78

Found: C, 61.53; H, 3.10; N, 4.72

25

### Example 63

7-Bromo-2-(4-hydroxy-2-methylphenyl)-1,3-benzoxazol-5-ol

The title compound was prepared in substantially the same manner as described in Example 21, from 4-methoxy-2-methylbenzoic acid, and 2-amino-6-bromo-4-methoxyphenol, and was obtained as a light purple solid, m.p. 120-135 °C; MS *m/e* 320 (M+H)<sup>+</sup>.

30

Analysis for: C<sub>14</sub>H<sub>10</sub>BrNO<sub>3</sub>

Calc'd: C, 52.52; H, 3.15; N, 4.38

Found: C, 52.24; H, 2.97; N, 4.15

**Example 64****2-(3-Fluoro-4-hydroxyphenyl)-7-(1-fluorovinyl)-1,3-benzoxazol-5-ol**

Hydrogen fluoride pyridine (1.14 mL) was added dropwise into a cold (0 °C) solution of 2-[4-(acetyloxy)-3-fluorophenyl]-7-vinyl-1,3-benzoxazol-5-yl acetate (0.25 g, 0.7 mmol), in sulfolane (3 mL). The reaction mixture was stirred for 5 min and then 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (120 mg) was added in one portion. The mixture was stirred at room temperature for 24 h, diluted with HCl (1N) and extracted with EtOAc. The organic layer was dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub> / isopropyl alcohol 0.3%) gave 7-(2-bromo-1-fluoroethyl)-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol as a white solid (0.25 g, m.p. 185-186°C). The product was taken in acetonitrile (2 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (150 mg) was added. The reaction mixture was stirred for 24 h, poured into cold (0°C) HCl (1N, 10 mL) and extracted with EtOAc. The organic extracts were dried over MgSO<sub>4</sub>. Evaporation and purification by flash chromatography (20% EtOAc / hexanes) gave a white solid (160 mg, m.p. 213-214°C); MS *m/e* 290 (M+H)<sup>+</sup>.

Analysis for: C<sub>15</sub>H<sub>9</sub>BrF<sub>2</sub>NO<sub>3</sub> x 0.3 H<sub>2</sub>O

Calc'd: C, 61.15; H, 3.28; N, 4.75

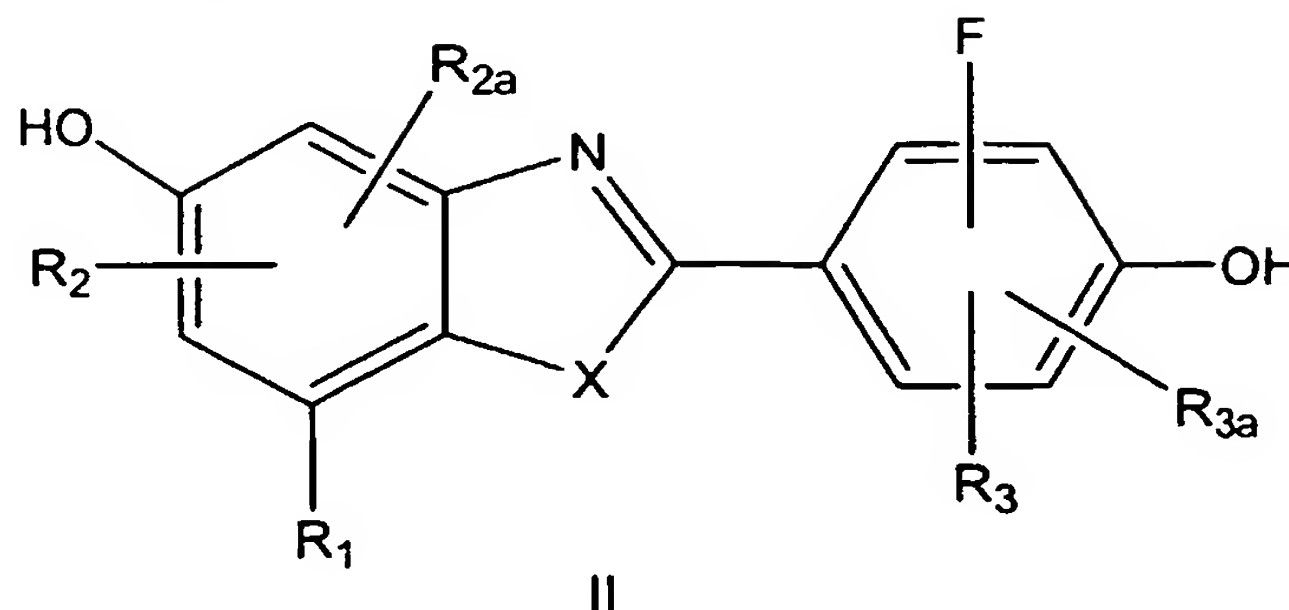
Found: C, 60.84; H, 3.41; N, 4.57

20



CLAIMS

1. A compound of formula II, having the structure



- 5 wherein

$R_1$  is alkenyl of 2-7 carbon atoms; wherein the alkenyl moiety is optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;

- 10  $R_2$  and  $R_{2a}$  are each, independently, hydrogen, hydroxyl, halogen, alkyl of 1-6 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl, alkenyl, or alkynyl moieties are optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;
- 15

- $R_3$ , and  $R_{3a}$  are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, halogen, alkoxy of 1-4 carbon atoms, trifluoroalkyl of 1-6 carbon atoms, or trifluoroalkoxy of 1-6 carbon atoms; wherein the alkyl, alkenyl, or alkynyl moieties are optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6 carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>, NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>;
- 20

$R_5$ ,  $R_6$  are each, independently hydrogen, alkyl of 1-6 carbon atoms or aryl of 6-10 carbon atoms;

X is O, S, or NR<sub>7</sub>;

- 25  $R_7$  is hydrogen, alkyl of 1-6 carbon atoms, aryl of 6-10 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub> or -SO<sub>2</sub>R<sub>5</sub>;

or a pharmaceutically acceptable salt thereof.

2. The compound according to claim 1, wherein X is O.
3. The compound according to claim 1 or 2, wherein  $R_1$  is alkenyl of 2-3 carbon  
5 atoms, which is optionally substituted with hydroxyl, -CN, halogen, trifluoroalkyl of 1-6  
carbon atoms, trifluoroalkoxy of 1-6 carbon atoms, -COR<sub>5</sub>, -CO<sub>2</sub>R<sub>5</sub>, -NO<sub>2</sub>, CONR<sub>5</sub>R<sub>6</sub>,  
NR<sub>5</sub>R<sub>6</sub> or N(R<sub>5</sub>)COR<sub>6</sub>.
4. The compound according to claim 1, which is 2-(3-fluoro-4-hydroxyphenyl)-7-  
10 vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.
5. The compound according to claim 1, which is 2-(2-fluoro-4-hydroxyphenyl)-7-  
vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.
- 15 6. The compound according to claim 1, which is 2-(2,3-difluoro-4-  
hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt  
thereof.
7. The compound according to claim 1, which is 4-bromo-2-(3-fluoro-4-  
20 hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt  
thereof.
8. The compound according to claim 1, which is 4,6-dibromo-2-(3-fluoro-4-  
hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt  
25 thereof.
9. The compound according to claim 1, which is 7-(1-bromovinyl)-2-(2-fluoro-4-  
hydroxyphenyl)-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.
- 30 10. The compound according to claim 1, which is 7-(1-bromovinyl)-2-(2,3-difluoro-  
4-hydroxyphenyl)-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.
11. The compound according to claim 1, which is 7-allyl-2-(3-fluoro-4-  
hydroxyphenyl)-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.

12. The compound according to claim 1, which is 2-(3,5-difluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.

5 13. The compound according to claim 1, which is 2-(3-fluoro-4-hydroxyphenyl)-7-(1-fluorovinyl)-1,3-benzoxazol-5-ol or a pharmaceutically acceptable salt thereof.

14. A compound which is,

- |    |    |  |
|----|----|--|
|    | a) | 2-(5-hydroxy-1,3-benzoxazol-2-yl) benzene-1,4-diol;            |
| 10 | b) | 3-(5-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol;             |
|    | c) | 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;              |
|    | d) | 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;              |
|    | e) | 2-(2-chloro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;              |
|    | f) | 2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol;              |
| 15 | g) | 2-(3- <i>tert</i> -butyl-4-hydroxyphenyl)-1,3-benzoxazol-6-ol; |
|    | h) | 2-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,4-diol;             |
|    | i) | 3-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol;             |
|    | j) | 4-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,2-diol;             |
|    | k) | 2-(3-chloro-4-hydroxyphenyl)-1,3-benzoxazol-6-ol;              |
| 20 | l) | 4-(5-hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol;             |
|    | m) | 4-(6-hydroxy-1,3-benzoxazol-2-yl)benzene-1,3-diol;             |
|    | n) | 6-chloro-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;     |
|    | o) | 6-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;      |
|    | p) | 6-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;              |
| 25 | q) | 5-chloro-2-(4-hydroxyphenyl)-1,3-benzoxazol-6-ol;              |
|    | r) | 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;      |
|    | s) | 7-bromo-2-(2-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;      |
|    | t) | 7-bromo-2-(2,3-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  |
|    | u) | 2-(4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol.;              |
| 30 | v) | 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  |
|    | w) | 7-(1-bromovinyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;      |
|    | x) | 7-ethynyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;             |
|    | y) | 2-(4-hydroxyphenyl)-7-propyl-1,3-benzoxazol-5-ol;              |
|    | z) | 7-butyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;               |

- 5 aa) 7-cyclopentyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  
 bb) ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate;  
 cc) 2-(4-hydroxyphenyl)-7-phenyl-1,3-benzoxazol-5-ol ;  
 dd) 2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol ;  
 ee) 7-ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol ;  
 ff) 7-ethyl-2-(2-ethyl-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  
 gg) 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carbaldehyde;  
 hh) 7-(hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  
 ii) 7-(bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol;  
 10 jj) [5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-yl] acetonitrile;  
 kk) 7-(1-hydroxy-1-methylethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol];  
 ll) 2-(4-hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol ;  
 mm) 2-(4-hydroxyphenyl)-7-isopropyl-1,3-benzoxazol-5-ol];  
 nn) 7-bromo-2-(4-hydroxy-3-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-ol;  
 15 oo) 7-(2-furyl)-2-(4-hydroxyphenyl) -1,3-benzoxazol-5-ol ;  
 pp) 2-(3-fluoro-4-hydroxyphenyl)- 7-(2-furyl)-1,3-benzoxazol-5-ol;  
 qq) 2-(4-hydroxyphenyl)- 7-thien-2-yl-1,3-benzoxazol-5-ol;  
 rr) 2-(4-hydroxyphenyl)-7-(1,3-thiazol-2-yl)-1,3-benzoxazol-5-ol;  
 ss) 2-(3-fluoro-4-hydroxyphenyl)-5-hydroxy-1,3-benzoxazole-7-carbonitrile;  
 20 tt) 4-bromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol ;  
 uu) 4,6-bibromo-2-(4-hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol ;  
 vv) 7-bromo-2-(3,5-difluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol;

or a pharmaceutically acceptable salt thereof.

25 15. A method of treating or inhibiting prostatitis or interstitial cystitis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

30 16. A method of treating or inhibiting inflammatory bowel disease, Crohn's disease, ulcerative proctitis, or colitis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

17. A method of treating or inhibiting prostatic hypertrophy, uterine leiomyomas, breast cancer, endometrial cancer, polycystic ovary syndrome, endometrial polyps, benign breast disease, adenomyosis, ovarian cancer, melanoma, prostate cancer, colon cancer, glioma or astioblastoma in a mammal in need thereof, which comprises  
5 providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

18. A method of lowering cholesterol, triglycerides, Lp(a), or LDL levels; inhibiting or treating hypercholesteremia; hyperlipidemia; cardiovascular disease;  
10 atherosclerosis; hypertension; peripheral vascular disease; restenosis, or vasospasm; or inhibiting vascular wall damage from cellular events leading toward immune mediated vascular damage in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

15 19. A method of providing cognition enhancement or neuroprotection; or treating or inhibiting senile dementias, Alzheimer's disease, cognitive decline, stroke, anxiety, or neurodegenerative disorders in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one  
20 of claims 1 to 13.

20. A method of treating or inhibiting free radical induced disease states in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

25 21. A method of treating or inhibiting vaginal or vulvar atrophy; atrophic vaginitis; vaginal dryness; pruritus; dyspareunia; dysuria; frequent urination; urinary incontinence; urinary tract infections in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one  
30 of claims 1 to 13.

22. A method of treating or inhibiting vasomotor symptoms in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

23. A method of inhibiting conception in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

5

24. A method of treating or inhibiting arthritis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

10

25. The method according to claim 24, wherein the arthritis is rheumatoid arthritis, osteoarthritis, or spondyloarthropathies.

15

26. A method of treating or inhibiting joint swelling or erosion; or treating or inhibiting joint damage secondary to arthroscopic or surgical procedures in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

20

27. A method of treating or inhibiting psoriasis or dermatitis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

25

28. A method of treating or inhibiting ischemia, reperfusion injury, asthma, pleurisy, multiple sclerosis, systemic lupus erythematosus, uveitis, sepsis, hemorrhagic shock, or type II diabetes in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

30

29. A method of treating or inhibiting endometriosis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a compound as claimed in any one of claims 1 to 13.

30. A pharmaceutical composition which comprises a compound as claimed in any one of claims 1 to 13 and a pharmaceutical carrier.



31. A method of treating or inhibiting inflammatory bowel disease in a mammal in need thereof, which comprises providing to said mammal an effective amount of an ER- $\beta$  selective ligand, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 20 times greater than its binding affinity to ER- $\alpha$ .

5

32. The method according to claim 31, wherein the inflammatory bowel disease is Crohn's disease, ulcerative colitis, indeterminate colitis, infectious colitis, or ulcerative proctitis.

10

33. The method according to claim 32, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 50 times greater than its binding affinity to ER- $\alpha$ .

15

34. The method according to claim 33, wherein the ER $\beta$  selective ligand causes an increase in wet uterine weight which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring uterotrophic activity, and the ER $\beta$  selective ligand causes an increase in casein kinase II mRNA which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring mammotrophic activity.

20

35. The method according to claim 34, wherein the ER- $\beta$  selective ligand does not significantly ( $p > 0.05$ ) increase wet uterine weight compared with a control that is devoid of uterotrophic activity, and does not significantly ( $p > 0.05$ ) increase casein kinase II mRNA compared with a control that is devoid of mammotrophic activity.

25

36. A method of treating or inhibiting arthritis in a mammal in need thereof, which comprises providing to said mammal an effective amount of a non-uterotropic, non-mammotrophic ER $\beta$  selective ligand, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 20 times greater than its binding affinity to ER- $\alpha$ .

30

37. The method according to claim 36, wherein the arthritis is rheumatoid arthritis, osteoarthritis, or spondyloarthropathies.

38. The method according to claim 37, wherein the binding affinity of the ER $\beta$  selective ligand to ER $\beta$  is at least about 50 times greater than its binding affinity to ER $\alpha$ .

5

39. The method according to claim 38, wherein the ER $\beta$  selective ligand causes an increase in wet uterine weight which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring uterotrophic activity, and the ER $\beta$  selective ligand causes an increase in casein kinase II mRNA which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring mammotrophic activity.

10

40. The method according to claim 39, wherein the ER $\beta$  selective ligand does not significantly ( $p > 0.05$ ) increase wet uterine weight compared with a control that is devoid of uterotrophic activity, and does not significantly ( $p > 0.05$ ) increase casein kinase II mRNA compared with a control that is devoid of mammotrophic activity.

15

41. A method of treating or inhibiting joint swelling or erosion; or treating or inhibiting joint damage secondary to arthroscopic or surgical procedures in a mammal in need thereof, which comprises providing to said mammal an effective amount of a non-uterotrophic, non-mammotrophic ER $\beta$  selective ligand, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 20 times greater than its binding affinity to ER- $\alpha$ .

20

25

42. The method according to claim 41, wherein the binding affinity of the ER $\beta$  selective ligand to ER $\beta$  is at least about 50 times greater than its binding affinity to ER $\alpha$ .

30

43. A method of treating or inhibiting endometriosis in a mammal in need thereof, which comprises providing to said mammal an effective amount of an ER- $\beta$  selective

ligand, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 20 times greater than its binding affinity to ER- $\alpha$ .

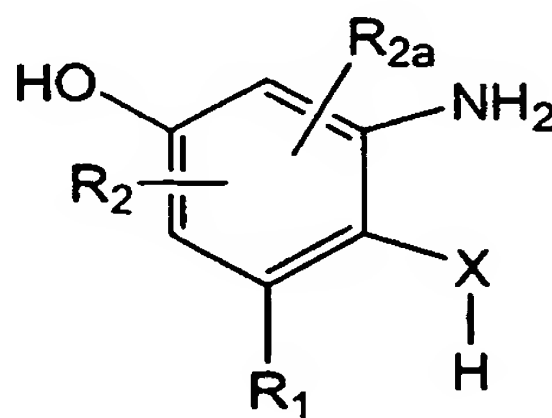
44. The method according to claim 43, wherein the binding affinity of the ER- $\beta$  selective ligand to ER- $\beta$  is at least about 50 times greater than its binding affinity to ER- $\alpha$ .

45. The method according to claim 44, wherein the ER $\beta$  selective ligand causes an increase in wet uterine weight which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring uterotrophic activity, and the ER $\beta$  selective ligand causes an increase in casein kinase II mRNA which is less than about 10% of that observed for a maximally efficacious dose of 17 $\beta$ -estradiol in a standard pharmacological test procedure measuring mammotrophic activity.

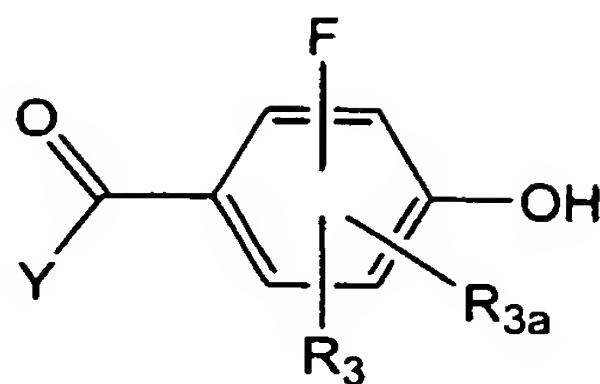
46. The method according to claim 45, wherein the ER- $\beta$  selective ligand does not significantly ( $p > 0.05$ ) increase wet uterine weight compared with a control that is devoid of uterotrophic activity and does not significantly ( $p > 0.05$ ) increase casein kinase II mRNA compared with a control that is devoid of mammotrophic activity.

47. A process for the preparation of a compound as claimed in any one of claims 1 to 13 comprising one of the following:

a) reacting a compound of formula



wherein  $R_1$ ,  $R_2$ ,  $R_{2a}$  and  $X$  are as defined in claim 1 with a compound of formula



wherein R<sub>3</sub> and R<sub>3a</sub> are as defined in claim 1 and Y is halogen, -OH or alkoxy of 1 to 6 carbon atoms;

or

- 5 b) converting a compound of formula II as defined in claim 1 to a pharmaceutically acceptable salt thereof;

or

- c) resolving an isomeric mixture of compounds of formula II to isolate an enantiomer of a compound of formula II or a pharmaceutically acceptable salt thereof.

10

## INTERNATIONAL SEARCH REPORT

Inter      at Application No  
PCT/US 02/38513

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7    C07D263/57    C07D277/66    C07D235/18    A61K31/4184    A61K31/423  
A61K31/428    A61P5/30    C07D413/04    C07D417/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7    C07D    A61K    A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, BEILSTEIN Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | STEVENS M F G ET AL: "Structural studies on bioactive compounds. 23. Synthesis of polyhydroxylated 2-phenylbenzothiazoles and a comparison of their cytotoxicities and pharmacological properties with genistein and quercetin"<br>JOURNAL OF MEDICINAL CHEMISTRY,<br>vol. 37, no. 11, 27 May 1994 (1994-05-27),<br>pages 1689-1695, XP002236361<br>the whole document, particularly tables 2 and 3 | 14                    |
| A          | the whole document, particularly page 1692, left-hand column, last paragraph  | 1-13,<br>15-47        |
| P, X       | ---<br>WO 02 051821 A (ASTRAZENECA AB)<br>4 July 2002 (2002-07-04)<br>the whole document<br>---   | 1-47                  |
| -/-        |   |                       |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

28 March 2003

Date of mailing of the international search report

09/04/2003

Name and mailing address of the ISA

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Allard, M

## INTERNATIONAL SEARCH REPORT

Inter      national Application No

PCT/US 02/38513

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| P,X        | WO 02 46168 A (ASTRAZENECA AB)<br>13 June 2002 (2002-06-13)<br>the whole document<br>---   | 1-47                  |
| A          | ANGERER, E VON ET AL:<br>"2-(Hydroxyphenyl)indoles: a new class of<br>mammary tumor inhibiting compounds"<br>JOURNAL OF MEDICINAL CHEMISTRY,<br>vol. 26, no. 1, January 1983 (1983-01),<br>pages 113-116, XP002236362<br>the whole document<br>---   | 1,15-47               |
| A          | ANGERER E VON ET AL: "2-Phenylindoles.<br>Relationship between structure, estrogen<br>receptor affinity, and mammary tumor<br>inhibiting activity in the rat"<br>JOURNAL OF MEDICINAL CHEMISTRY,<br>vol. 27, no. 11, November 1984 (1984-11),<br>pages 1439-1447, XP002236363<br>the whole document<br>----- | 1,15-47               |



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 02/38513

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 15-29 and 31-46 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 31-46 (all in part)  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 31-46 (all in part)

Present claims 31-46 relate to methods using compounds defined by reference to a desirable property, namely their ER-beta binding selectivity.

The claims cover methods using all compounds having this property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, said claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product to be used by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of claims 31-46 which appear to be clear, supported and disclosed, namely those parts relating to the methods using compounds as defined in claims 1-13.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 02/38513

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s) |             | Publication<br>date |
|---|---|---------------------|----------------------------|-------------|---------------------|
| WO 02051821                               | A | 04-07-2002          | WO                         | 02051821 A1 | 04-07-2002          |
| WO 0246168                                | A | 13-06-2002          | AU                         | 2123902 A   | 18-06-2002          |
|   |   |                     | WO                         | 0246168 A1  | 13-06-2002          |